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Scope

The Atlas of Genetics and Cytogenetics in Oncology and Haematology is a peer reviewed on-line journal in open access, devoted to genes, cytogenetics, and clinical entities in cancer, and cancer-prone diseases. It presents structured review articles (“cards”) on genes, leukaemias, solid tumours, cancer-prone diseases, and also more traditional review articles (“deep insights”) on the above subjects and on surrounding topics. It also present case reports in hematology and educational items in the various related topics for students in Medicine and in Sciences.

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# Table of contents

## Gene Section

<table>
<thead>
<tr>
<th>Gene</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AURKB</strong> (aurora kinase B)</td>
<td>872</td>
</tr>
<tr>
<td>Sai-Ching Jim Yeung</td>
<td></td>
</tr>
<tr>
<td><strong>CD74</strong> (CD74 molecule, major histocompatibility complex, class II invariant chain)</td>
<td>879</td>
</tr>
<tr>
<td>Naama Gil-Yarom, Shirly Becker Herman, Idit Shachar</td>
<td></td>
</tr>
<tr>
<td><strong>ETV6</strong> (ets variant 6)</td>
<td>886</td>
</tr>
<tr>
<td>Etienne De Braekeleer, Nathalie Douet-Guilbert, Marc De Braekeleer</td>
<td></td>
</tr>
<tr>
<td><strong>EZH2</strong> (enhancer of zeste homolog 2 (Drosophila))</td>
<td>900</td>
</tr>
<tr>
<td>Amir Avan, Mina Maftouh, Hamid Fuji, Elisa Giovannetti, Godefridus J Peters</td>
<td></td>
</tr>
<tr>
<td><strong>HSPB8</strong> (heat shock 22kDa protein 8)</td>
<td>907</td>
</tr>
<tr>
<td>Dominique Bollino, Laure Aurelian</td>
<td></td>
</tr>
<tr>
<td><strong>PIWIL2</strong> (piwi-like RNA-mediated gene silencing 2)</td>
<td>919</td>
</tr>
<tr>
<td>Jian-Xin Gao, Ning Liu, Hai-Long Wu</td>
<td></td>
</tr>
<tr>
<td><strong>ABCC10</strong> (ATP-binding cassette, sub-family C (CFTR/MRP), member 10)</td>
<td>928</td>
</tr>
<tr>
<td>Elizabeth Hopper-Borge, Natalya Domanitskaya</td>
<td></td>
</tr>
<tr>
<td><strong>CHST11</strong> (carbohydrate (chondroitin 4) sulfotransferase 11)</td>
<td>932</td>
</tr>
<tr>
<td>Michael Klüppel</td>
<td></td>
</tr>
<tr>
<td><strong>POU3F2</strong> (POU class 3 homeobox 2)</td>
<td>938</td>
</tr>
<tr>
<td>Takuya Yazawa</td>
<td></td>
</tr>
<tr>
<td><strong>PRAME</strong> (preferentially expressed antigen in melanoma)</td>
<td>941</td>
</tr>
<tr>
<td>Joel Fulton, David M Heery</td>
<td></td>
</tr>
</tbody>
</table>

## Leukaemia Section

<table>
<thead>
<tr>
<th>Karyotype and Genes</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>dic(9;16)(p13;q11) PAX5/?</td>
<td>945</td>
</tr>
<tr>
<td>Jean-Loup Huret</td>
<td></td>
</tr>
<tr>
<td>dic(9;17)(p13;q11) PAX5/TAOK1</td>
<td>947</td>
</tr>
<tr>
<td>Jean-Loup Huret</td>
<td></td>
</tr>
<tr>
<td>t(3;12)(q26;p13) ETV6/MECOM</td>
<td>950</td>
</tr>
<tr>
<td>Etienne De Braekeleer, Nathalie Douet-Guilbert, Marc De Braekeleer</td>
<td></td>
</tr>
<tr>
<td>t(6;20)(q13;q12) LMBRD1/CHD6</td>
<td>954</td>
</tr>
<tr>
<td>Nathalie Douet-Guilbert, Etienne De Braekeleer, Corinne Tous, Nadia Guéganic, Frédéric Morel, Marc De Braekeleer</td>
<td></td>
</tr>
</tbody>
</table>

## Deep Insight Section

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted human structural clusters of miRNAs target cancer genes</td>
<td>957</td>
</tr>
<tr>
<td>Anthony Mathelier, Alessandra Carbone</td>
<td></td>
</tr>
</tbody>
</table>
AURKB (aurora kinase B)

Sai-Ching Jim Yeung

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Abstract

Review on AURKB, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: AIK2, AIM-1, AIM1, ARK2, AurB, IPL1, PPP1R48, STK12, STK5, aurkb-sv1, aurkb-sv2

HGNC (Hugo): AURKB

Location: 17p13.1

DNA/RNA

Description

Aurora kinases are conserved during eukaryotic evolution. While the genomes of yeasts encode only one Aurora kinase (Ipl1 in budding yeast and Ark1 in fission yeast), higher eukaryotes express two or more members of this family. In mammals, there are three members (Aurora A, B and C).
Aurora A (Aurora 2, ARK1, and BTAK) is present among all vertebrates. Aurora B (Aurora 1, ARK2, and AIM1) and Aurora-C (Aurora 3 and AIK3) came into existence from gene duplication during the evolution of mammals (Brown et al., 2004).

**Protein**

**Description**

Size: 344 amino acids; molecular weight: 39.3 kDa. The AURKB gene has 8 exons, but there are 4 isoforms of AURKB protein due to alternative splicing.

The molecular structure of Aurora B has been determined by crystallography (Sessa et al., 2005; Andersen et al., 2008; Girdler et al., 2008). When the amino acid sequences of the ATP-binding pockets of Aurora A, B and C are compared, 3 of the 26 residues lining the active site in Aurora A, Leu215, Thr217 and R220, are different from the corresponding residues in Aurora B and C (Brown et al., 2004); there is no difference in these 26 residues between Aurora B and C. The manner in which Aurora A binds to ATP (Bayliss et al., 2003) and ADP (Nowakowski et al., 2002) have been modeled. Because of the high degree of conservation of the active site between Aurora A and B, Aurora B is expected to interact with ATP and ADP in the same way as reported for Aurora A. INCENP interacts with AURKB at an α-helix domain and activates AURKB. AURKB-Sv1 lacks the INCENP interaction domain because part of exon 5 is missing and intron 5 and intron 6 may form a new domain. Therefore, the conformation of AURKB-Sv1 is expected to be different from AURKB, and the interaction with INCENP will be affected.

In contrast, the α-helix domain for interacting with INCENP is intact in AURKB-Sv2, but AURKB-Sv2 is missing major parts of the kinase domain. Thus, AURKB-Sv2 does not have kinase activity and may act as a dominant negative competitor against AURKB.

Small molecule inhibitors target the ATP-binding pocket of aurora kinases. There are data available from the Protein Data Bank website (http://www.pdb.org) regarding the interaction of Aurora B with 3 inhibitors [AD6 (4-[(5-bromo-1,3-thiazol-2-yl) amino]-N-methylbenzamide): PDB ID 2vgp (Andersen et al., 2008), ZM447439: PDB ID 2vrx (Girdler et al., 2008), and hesperadin: PDB ID 2bfy (Sessa et al., 2005)].

**Expression**

The expression of Aurora kinases varies with cell cycle phases, being at very low levels in the non-M phases (Fu et al., 2007; Gautschi et al., 2008; Mountzios et al., 2008). Although Aurora A and Aurora B are phylogenetically related, they have different biological functions and distinct temporospatial subcellular localization in mammals.

In prophase, Aurora A is at the centrosomes while Aurora B starts to appear in the nucleus (Carmena and Earnshaw, 2003). In metaphase, Aurora A is near the spindle poles on the microtubules ends while Aurora B is on the centromeric regions of chromosomes as a chromosomal passenger protein.
AURKB (aurora kinase B) Yeung SCJ

AURKB has 4 alternative splicing variants: the longest one (344 aa); AURKB-Sv1 (312 aa), which loses part of exon 5 but contains a fragment of intron 5 and intron 6 instead; AURKB-Sv2 (303 aa), which lacks exon 6; and the shortest form (142 aa), which loses more than the C-terminal half of the protein (López-Saavedra and Herrera, 2010). The serines and threonines are highlighted to demonstrate the alignment. Helix domains are highlighted in magenta. The active site is highlighted in red. The nucleotide binding region is indicated by the rectangle.

The surface model of ZM447439 bound to Aurora B (amino acid 77-361) is rendered using the program PyMOL. ZM447439 (in stick molecular structure) is shown in the active site ATP-binding pocket of Aurora B (blue), and the 3 residues that differ between Aurora B and A are highlighted in cyan. INCENP-A (amino acid 798-840) in complex with Aurora B is hidden by coloring it black.
In anaphase, Aurora A is at the polar microtubules while Aurora B relocates from the centromeres to the spindle midzone (spindle equator) where the microtubules from opposite poles interdigitate (Keen and Taylor, 2004). In cytokinesis, Aurora B accumulates at the cleavage furrow before finally concentrating at the midbody.

**Localisation**

The protein level and kinase activity of Aurora B are tightly controlled according to the phase of the cell cycle and the stages in mitosis. It expression peaks at the G2-M transition, whereas its kinase activity and subcellular localization change quickly according to the stage of mitosis. In mitosis, Aurora B, Survivin, Borealin and INCENP form the chromosomal passenger complex (Knauer et al., 2007). It is located at the chromosomes in prophase, the centromeres in prometaphase and metaphase, the central mitotic spindle in anaphase, and the cleavage furrow in cytokinesis (Adams et al., 2001). Analysis of the dynamics of Aurora B showed that the association of Aurora B with centromeres is dynamic (Murata-Hori et al., 2002) but the association with spindle microtubules during anaphase is static. Aurora B is transported to the equatorial cell cortex by astral microtubules.

**Function**

In anaphase, Aurora A is at the polar microtubules while Aurora B relocates from the centromeres to the spindle midzone (spindle equator) where the microtubules from opposite poles interdigitate (Keen and Taylor, 2004). In cytokinesis, Aurora B accumulates at the cleavage furrow before finally concentrating at the midbody.

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like or B-like functions and properties (Fu et al., 2009).

**Implicated in**

**Lung adenocarcinoma**

**Note**
(Smith et al., 2005; Vischioni et al., 2006)

**Prognosis**
High Aurora B expression predicts short survival for lung adenocarcinoma (Vischioni et al., 2006).

**Oncogenesis**
High Aurora B expression correlates with cell proliferation, dedifferentiation, and metastasis in lung cancer patients (Vischioni et al., 2006).

**Acute myeloid leukemia**

**Note**
(Ikezoe et al., 2007)

**Oncogenesis**
In an analysis comparing 44 samples of freshly isolated AML cells with 11 bone marrow mononuclear cells from healthy volunteers and 12 peripheral blood mononuclear cells from healthy volunteers, measurement of relative mRNA expression of Aurora B shows that it is upregulated in the majority of cases (Ikezoe et al., 2007). In high-risk myelodysplastic syndrome and secondary AML, surviving and aurora B are expressed in high levels in the CD34+ cells (Yoshida et al., 2012). Aurora B overexpression may have contributed to genomic instability and progression from myelodysplastic syndrome to AML.

**Prostate cancer**

**Note**
(Ditchfield et al., 2003; Chieffi et al., 2006)

**Oncogenesis**
Aurora B expression correlates with prostate cancer cell proliferation (Chieffi et al., 2006).

**Anaplastic thyroid carcinomas**

**Note**
(Sorrentino et al., 2005)

**Oncogenesis**
Aurora B overexpression promotes thyroid carcinoma cell proliferation and is associated with undifferentiated thyroid cancer (Sorrentino et al., 2005).

**Hepatocellular carcinoma**

**Note**
(Sistayanarain et al., 2006)

**Prognosis**
High Aurora B expression predicts short survival for hepatocellular carcinoma (Tanaka et al., 2008).

Yasen et al. (Yasen et al., 2009) studied the expression of the isoforms of Aurora B in 10 HCC cell lines and 253 samples from patients with varying degrees of HCC malignancy. AURKB was aberrantly expressed in HCC cell lines and primary HCC tumors.

**Abnormal protein**
Expression of AURKB-Sv2 is associated with advanced stages of HCC, high levels of α-fetoprotein, tumor invasion, multiple tumor formation, and shortened disease-free survival (Yasen et al., 2009). AURKB-Sv2 may be a marker of poor prognosis.

**Oncogenesis**
Cell cycle phase-inappropriate phosphorylation of histone H3 in the entire cell cycle may enhance proliferation of liver cancer cells (Sistayanarain et al., 2006).

**Glioblastoma multiforme**

**Note**
(Zeng et al., 2007)

**Prognosis**
High Aurora B expression predicts short survival for glioblastoma multiforme (Zeng et al., 2007).

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AURKB (aurora kinase B)
Yeung SCJ


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This article should be referenced as such:
CD74 (CD74 molecule, major histocompatibility complex, class II invariant chain)

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Abstract

Review on CD74, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: DHLAG, HLADG, II, Ia-GAMMA
HGNC (Hugo): CD74
Location: 5q32

DNA/RNA

Description

The CD74 gene consists of 9 exons, different transcripts results in four protein variants.

Transcription

CD74 gene is processed into 4 different in-vivo know transcripts resulting from two different translation initiation sites and alternative splicing (Borghese and Clanchy, 2011; UniProt Consortium, 2013):

P43 - The longest isoform. Contains a longer cytoplasmic tail due to the use of an alternative translation initiation site, and a THY domain from alternative splicing.
P41 - Similar to the P43 isoform but does not contain the longer cytoplasmic tail.
P35 - Similar to the P43 isoform but does not contain the THY domain.
P33 - Does not contain not the longer cytoplasmic tail and not the THY domain.
CD74 (CD74 molecule, major histocompatibility complex, class II invariant chain) Gil-Yarom N, et al.

Protein

**Description**
CD74 is a non-polymorphic type II integral membrane protein. The most common isoform is p33, which is 296 aa long and has a molecular weight of 33 kDa. The protein consists of three parts, a 46 aa long N-terminus cytoplasmic tail, 26 aa long transmembranal domain and a 224 aa long luminal region. CD74 assembles to homotrimers immediately after synthesis.

**Expression**
CD74 is mainly expressed in antigen presenting cells, endothelial cells and neuroglia cells.

**Localisation**
Trimers of CD74 are expressed in the endoplasmic reticulum (ER), in association with MHC α and β chains. The complex is transported to the trans-Golgi and then diverted from the secretory pathway to the endocytic system and ultimately to acidic endosome or lysosome-like structures called MHC class II compartments (MIIC or CIIV). A small proportion of CD74 is modified by the addition of chondroitin sulfate (CD74-CS), and this form of CD74 is expressed on the cell surface (Stumptner-Cuvelette and Benaroch, 2002).

**Function**
CD74 function has two main functions:

1) **MHCII chaperon**

MHC class II molecules are heterodimeric complexes that present foreign antigenic peptides on the cell surface of antigen-presenting cells (APCs) to CD4+ T cells. MHC class II synthesis and assembly begins in the endoplasmic reticulum (ER) with the non-covalent association of the MHC α and β chains with trimers of CD74. Three MHC class II α β dimers bind sequentially to a trimer of the CD74 to form a nonameric complex (αβCD74)3, which then exits the ER (Roche et al., 1991). After being transported to the trans-Golgi, the αβCD74 complex is diverted from the secretory pathway to the endocytic compartments. Once in the endocytic compartments, CD74 is proteolytically processed. CD74 luminal domain undergoes a stepwise proteolytic cleavage, which results in a short class II-associated Ii chain peptide (CLIP), which remains in the MHC class II peptide groove (Neeffjes et al., 1990; Roche and Cresswell, 1991; Stumptner-Cuvelette and Benaroch, 2002). The final step for MHC class II expression requires interaction of αβCLIP complexes with another class II-related αβ dimer, called HLA-DM. Binding of this molecule excludes the residual CLIP peptide, rendering the αβ dimers ultimately competent to bind antigenic peptides, which are mainly derived from internalized antigens and also are delivered to the endocytic pathway (Denzin and Cresswell, 1995; Ghosh et al., 1995). Thus, CD74 was thought to function mainly as MHC class II chaperone, which promotes ER exit of MHC class II molecules, directs them to endocytic compartments, prevents self-peptide binding in the ER and contributes to peptide editing in the MHC class II compartment (Matza et al., 2003).

2) **CD74 as cell surface receptor**

A small proportion of CD74 is modified by the addition of chondroitin sulfate (CD74-CS), and this form of CD74 is expressed on the cell surface (Matza et al., 2003; Naujokas et al., 1993). This cell surface expression of CD74 is not strictly dependent on class II MHC (Henne et al., 1995; Starlets et al., 2006), and numerous non-class II positive cells express CD74 where it can serve as a receptor for the initiation of different signaling cascades (Maharshak et al., 2010; Stumptner-Cuvelette and Benaroch, 2002). The cytokine, macrophage migration inhibitory factor (MIF), was found to be the natural ligand of CD74. MIF binds to the extracellular domain of CD74 with high affinity (KD = 1.40 × 10^-9 M) and initiates a signaling cascade (Leng et al., 2003). CD74 forms a complex with CD44, which is essential for the MIF-induced signaling cascade (Gore et al., 2008; Shi et al., 2006).

In murine B cells, CD74 expression is directly involved in shaping the B cell repertoire by regulating mature B cell survival (Gore et al., 2008; Matza et al., 2003; Shachar and Flavell, 1996; Starlets et al., 2006). MIF binding to CD74 induces a signaling pathway that involves the Syk tyrosine kinase and the PI3K/Akt pathway (Gore et al., 2008; Starlets et al., 2006), induction of CD74 intramembrane cleavage, and the release of the CD74 intracellular domain (CD74-ICD) (Matza et al., 2002; Schneppenheim et al., 2013). CD74-ICD translocates to the nucleus where it induces activation of transcription mediated by the NF-κB p65/RelA homodimer and its co-activator, TAFII105, resulting in regulation of transcription of
genes that control B cell proliferation and survival (Gore et al., 2008; Matza et al., 2001; Starlets et al., 2006). MIF was found to regulate cell entry into the S-phase in a CD74 and CD44-dependent fashion, by elevating cyclin E levels, resulting in cell proliferation. In addition, this cascade augments Bcl-2 expression, further supporting cell survival (Cohen et al., 2012; Gordin et al., 2010; Gore et al., 2008; Lantner et al., 2007; Sapoznikov et al., 2008; Starlets et al., 2006). Thus, the MIF binding to CD74/CD44 complex initiates a pathway, resulting in proliferation of the mature B cell population, and their rescue from death.

**Implicated in**

**Chronic lymphocytic leukaemia (CLL)**

**Prognosis**

CD74 and its ligand, MIF, were shown to play a pivotal role in the regulation of CLL cell survival. CLL cells markedly upregulate both expression of their cell surface CD74, and their MIF production. Stimulation of CD74 with the MIF ligand (as well as with an agonistic antibody) initiates a signaling cascade leading to IL-8 transcription and secretion in all CLL cells, regardless of the clinical status of the patients. Secreted IL-8 induces the transcription and translation of the anti-apoptotic protein, Bcl-2, and thus regulates an anti-apoptotic pathway. Blocking of CD74 (by milatuzumab), or of MIF or IL-8 results in dramatic downregulation of Bcl-2 expression, and augmentation of apoptosis (Binsky et al., 2007).

In addition, stimulation of CD74 with its natural ligand, MIF, initiates a signaling cascade that results in upregulation of TAp63, which directly regulates CLL survival. TAp63 expression also elevates the expression of the integrin VLA-4, particularly during the advanced stage of the disease. Blocking of CD74, TAp63, or VLA-4 inhibits the in vivo homing of CLL cells to the bone marrow (BM). Thus, CD74 and its target genes TAp63 and VLA-4 facilitate migration of CLL cells back to the BM, where they interact with the supportive BM environment that rescues them from apoptosis (Binsky et al., 2010).

**Multiple myeloma (MM)**

**Prognosis**

CD74 expression was observed in 19 of 22 cases of multiple myeloma, with most expressing moderate to high levels in the majority of malignant plasma cells (Burton et al., 2004). CD74, expressed in MM, was evaluated as a target for immunotherapy with milatuzumab (a humanized anti-CD74 antibody). In a multicentre dose escalation study, 25 patients with advanced MM received milatuzumab doses of 1.5 (N = 8), 4.0 (N = 9), 8.0 (N = 4) or 16.0 mg/kg (N = 4) administered twice weekly x 4.

**CD74 activation by MIF up regulates cell survival and VLA-4 expression.**

They had a median of 5 prior treatments (17 post ≥ 1 stem cell transplantation) and were refractory (N = 7) or relapsed (N = 18) with generally short-lived responses to last treatment (median 4.0 months). After increasing prophylactic medications and slowing administration, infusions were well tolerated (National Cancer Institute-Common Terminology Criteria v3 toxicity Grades 1-2) with no dose-limiting toxicity at higher doses. Only one patient developed borderline positive human anti-milatuzumab antibody titres of uncertain clinical significance. Although milatuzumab was rapidly cleared from circulation with little serum accumulation and low trough levels, B-cell levels were moderately decreased with treatment (median decrease, 34%). Disease stabilization and evidence of pharmacodynamic activity support further development for use in combination with other agents or as a drug conjugate (Kaufman et al., 2013).

**Mantle cell lymphoma (MCL)**

**Prognosis**

CD74 is expressed on MCL. The combination of milatuzumab and rituximab has preclinical in vitro and in vivo activity in MCL (Alinari et al., 2011). Treatment of MCL cell lines and primary patient tumor cells with immobilized milatuzumab resulted in statistically significant enhanced cell death (Alinari et al., 2012).

**Non-Hodgkin lymphoma (NHL)**

**Prognosis**

Preclinical studies of the humanized anti-CD74 mAb hLL1 have shown that it is an effective therapeutic agent that may be of significant value for treatment of NHL (Stein et al., 2007).
**Invasive carcinoma of the bladder**

**Prognosis**
CD74 expression is increased in high-grade, invasive carcinoma of the bladder. Its expression was significantly associated with older age at diagnosis (Choi et al., 2013).

**Gastrointestinal carcinoma**

**Prognosis**
Expression of CD74 within gastrointestinal carcinomas showed a statistically greater expression than in the normal tissue counterparts. CD74 expression was observed in 95% of pancreatic carcinomas with the majority of cases presenting a mostly intense, diffuse labeling pattern. The results suggested a trend towards greater expression within the higher-grade carcinomas. Colorectal and gastric carcinomas gave similar results with 60% and 86%, respectively, positive for CD74 with an intense, diffuse staining pattern. For PanIN lesions there was greater expression of CD74 within higher grade, PanIN-3 lesions, whereas the colonic adenomas showed no such trend, but overall, a higher frequency and intensity of CD74 labeling than was observed within the colon carcinomas. These findings are supportive of a role for CD74 in the development and maintenance of gastrointestinal neo-plasia, and provide a rationale for development of therapeutic agents that are able to block CD74 function, specifically within the tumor cell (Gold et al., 2010).

**Non-small cell lung cancer**

**Prognosis**
CD74 was found to be expressed on non-small cell lung cancer (NSCLC) cells (Ioachim et al., 1996). CD74 immunoreactivity was present in the stromal cells in most tumors. However, in many tumors the malignant cells themselves also strongly expressed CD74 (McClelland et al., 2009).

**Thymic epithelial neoplasms**

**Prognosis**
Sixty-four thymic epithelial neoplasms (27 cases of benign thymoma, 20 cases of invasive thymoma, and 17 cases of true thymic carcinoma) were studied for neoplastic epithelial cell expression of CD74 and MHC class II molecules by immunohistochemical staining of paraffin-embedded tissue. Neoplastic epithelial cells in 88% of thymic carcinomas (15/17), 70% of invasive thymomas (14/20), but only 33% of benign thymomas (9/27) were immunoreactive for CD74. A subset of CD74-positive neoplasms was positive for MHC class II as well, with higher relative rates of dual positivity in more aggressive neoplasms. In addition, specific histologic subtypes of thymic epithelial neoplasms displayed differing patterns of CD74 positivity. Based on these findings, CD74 and MHC class II are useful markers for the classification of thymic epithelial neoplasms (Datta et al., 2000).
**Pancreatic cancer**

**Prognosis**

Pancreatic ductal adenocarcinoma (PDAC) is still one of the most fatal cancers. Sixty-eight patients receiving curative extended resection combined with preoperative chemoradiation and postoperative chemotherapy for primary PDAC were selected. Immunohistochemistry using anti-CD74 antibody on paraffin-embedded tissue samples was performed, and cases were divided into two groups according to the ratio of CD74-positive cells: expression level I, CD74-positive cells or=70%. The correlation of CD74 expression level with clinicopathological features and overall survival was evaluated. Forty-seven (69.1%) and 21 (30.9%) patients showed level I and II CD74 expression, respectively. Patients with level I CD74 expression had a significantly better survival rate than those with level II (P = 0.003). Among the patients with pathological tumor-node-metastasis stages I and II, those with level I CD74 expression showed a significantly better prognosis than those with level II (P = 0.006). CD74 expression proved as a useful prognostic indicator for PDAC treated with multimodal therapy (Nagata et al., 2009).

**Atherosclerosis**

**Prognosis**

Overexpression of CD74 has been reported in atherosclerotic plaques. Stimulation of CD74 with an anti-CD74 antibody, which binds the CD74 extracellular domain, induces the expression of the NF-κB-regulated gene MCP-1, a small cytokine that belongs to the CC chemokine family. MCP-1 recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation (Martín-Ventura et al., 2009).

**Alzheimer**

**Prognosis**

CD74 has been found to be upregulated in the microglia and neurons of Alzheimer's patients and can interact with the amyloid precursor protein, potentially inhibiting the production of amyloid-β.

**HIV**

**Prognosis**

HIV-1 gp41 binds directly to CD74 in HIV-1-infected cells, leading to ERK1/ERK2 MAPK activation and enhanced HIV-1 infection (Zhou et al., 2011). The cytoplasmic region of HIV-1 Vpu also was found to interact with the 30-amino-acid cytoplasmic tail of CD74. Human monocytic U937 cells infected with wild-type or Vpu-defective HIV-1 showed that Vpu down-regulated the surface expression of MHC class II molecules (Hussain et al., 2008).

**Gastric ulceration**

**Prognosis**

The pathogenic bacterium, Helicobacter pylori, was shown to bind to CD74 on gastric epithelial cells. Upon H. pylori binding to CD74, NF-κB activation occurs resulting in the production of proinflammatory cytokines, including IL-8. IL-8 plays a major role in the proinflammatory immune response to H. pylori infection, and the interaction of H. pylori with the gastric epithelial cells might be of critical importance in the immune response to this infection and the development of gastric ulceration (Beswick et al., 2005).

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Abstract
The ETV6 gene located at band 12p13 encodes a protein containing two major domains, the HLH (helix-loop-helix) domain, encoded by exons 3 and 4, and the ETS domain, encoded by exons 6 through 8, with in between the internal domain encoded by exon 5. ETV6 is a strong transcriptional repressor, acting through its HLH and internal domains. Five potential mechanisms of ETV6-mediated carcinogenesis have been identified: constitutive activation of the kinase activity of the partner protein, modification of the original functions of a transcription factor, loss of function of the fusion gene, affecting ETV6 and the partner gene, activation of a proto-oncogene in the vicinity of a chromosomal translocation and dominant negative effect of the fusion protein over transcriptional repression mediated by wild-type ETV6. Thirty-three ETV6 partner genes have been identified.

Identity
Other names: TEL, TEL1
HGNC (Hugo): ETV6
Location: 12p13.2

DNA/RNA
Description
A member of the ets (E-26 transforming specific) family of transcription factors; the gene spans a region of 240 kb and consists of 8 exons. There are two start codons, one (exon 1a starting at codon 1) located at the beginning of the gene and another alternative (exon 1b starting at codon 43) upstream of exon 3.

Transcription
Transcription is from telomere to centromere; there are three species of transcripts : 2400 kb, 4300 kb and 6200 kb; the gene encodes for a 1356 kb cDNA.
**Protein**

**Description**
The ETV6 protein is a 452 amino acid polypeptide that shares homology at the 5' and 3' ends with other ETS family members. ETS proteins form one of the largest families of signal-dependent transcriptional regulators, mediating cell proliferation, differentiation and tumorigenesis. The ETV6 protein contains two major domains, the HLH (helix-loop-helix) and ETS domains. The HLH domain, also referred to as the pointed or sterile alpha motif domain, is encoded by exons 3 and 4 and is responsible for hetero- and homodimerization with other ETV6 proteins and possibly other ets family members. The ETS domain, encoded by exons 6 through 8, is responsible for sequence specific DNA-binding and protein-protein interaction. A central domain, called internal domain, is encoded by exon 5 and is involved in the recruitment of a repression complex including N-Cor, mSin3 and SMRT.

**Expression**
Expression arrays and Northern analysis have shown ubiquitous expression with greater expression in bone marrow, spleen and thymus.

**Localisation**
Immunofluorescence has shown a nuclear localization.

**Function**
The ETV6 protein plays a crucial role in the embryonic development and hematopoietic regulation.

ETV6 is essential for normal development and is specifically required for maintaining blood vessel integrity within the developing yolk sac and survival of different cell types in the developing embryo. ETV6 is essential for the establishment of hematopoiesis of all lineages in the bone marrow. ETV6 is a strong transcriptional repressor. Repression is mediated by the HLH domain and the internal domain. Repression by the HLH domain is mediated through interaction with the HLH domain of L3MBTL1, a member of the polycomb group of chromatin-associated proteins, that can maintain long term repression of genes through a histone deacetylase-independent mechanism. Repression by the internal domain is mediated through interaction with corepressors such as N-Cor, mSin3 and SMRT, which in turn can recruit histone deacetylases.

**Mutations**

**Note**
ETV6 is implicated in leukemia, myelodysplastic syndromes and sarcoma.

**Deletions:** ETV6 is frequently deleted in hematological malignancies. The deletion of the normal (untranslocated) ETV6 allele in the presence of a translocation affecting ETV6 is quite frequent, notably in patients with ETV6-RUNX1, ETV6-NTRK3, ETV6-ABL1, ETV6-ACSL6 and ETV6-STL fusion. Deletion of an ETV6 allele has also been observed in the absence of rearrangement of the second allele.
## Implicated in

### t(1;12)(p36;p13) MDS2/ETV6

**Disease**
One CML with t(9;22) (no molecular analysis) and one refractory anemia with excess of blasts in transformation.

**Abnormal protein**

**Oncogenesis**
Loss of function of ETV6? Expression of RPL11, centromeric to MDS2 (63.5 kb) much higher in the patient than in controls.

### t(1;12)(q21;p13) ARNT/ETV6

**Disease**
One AML-M2 and one T-cell acute lymphoblastic leukemia.

**Hybrid/Mutated gene**
The ETV6-ARNT transcript contains the first 4 exons of ETV6 fused in frame with exon 1 or 2 of ARNT.

**Abnormal protein**
The ETV6-ARNT protein contains the oligomerization domain of ETV6 and almost all of the ARNT protein, including its major domains.

**Oncogenesis**
Given the presence of the oligomerization domain of ETV6 in the ETV6-ARNT protein, it is expected that the HLH domain of ETV6 convert ARNT from a transcriptional activator into a repressor. Furthermore, the ETV6-ARNT fusion protein retaining the HLH domain of ETV6 could interact with the other ETV6 protein.

### t(1;12)(q25;p13) ABL2/ETV6

**Disease**
AML-M3, AML-M4, T-cell ALL, B-cell ALL.

**Hybrid/Mutated gene**
Breakpoint in intron 5 of ETV6 in all three cases.

**Abnormal protein**
Fusion protein contains the HLH oligomerization domain of ETV6 and the SH2, SH3, and protein tyrosine kinase domains of ABL2.

**Oncogenesis**
Constitutive activation of the kinase activity of ABL2.

### t(3;12)(q26;p13) MDS1-EVI1 (MECOM)/ETV6

**Note**
Rare, but recurrent, chromosomal aberration (more than 30 cases). Few patients studied on a molecular level.

**Disease**
Myeloproliferative disorders, myelodysplastic syndromes and acute myelogenous leukemia.

**Hybrid/Mutated gene**
Two different mechanisms for generating the fusion gene.
- First mechanism: in-frame chimeric transcript consisting of the first two exons of ETV6 fused to MDS1 sequences, which in turn is fused to the second exon of the EVI1 gene.
- Second mechanism: direct fusion between ETV6 and EVI1, in which case an out-of-frame fusion between exon 2 of ETV6 and exon 2 of EVI1 is generated but the open reading frame of EVI1 is not disrupted.

**Abnormal protein**
In both fusion types, ETV6 contributes no known functional domain to the predicted chimeric protein.

**Oncogenesis**
Oncogenic potential of the translocation could be the result of the ETV6 promoter driving the transcription of EVI1, resulting in activation of the transcription factor EVI1, which is not normally expressed in hematopoietic cells.

### t(4;12)(p16;p13) FGFR3/ETV6

**Disease**
Peripheral T-cell lymphoma

**Hybrid/Mutated gene**
Fusion of exon 5 of ETV6 to exon 10 of FGFR3.

**Abnormal protein**
Protein consists of the HLH domain of ETV6 and the tyrosine kinase domain of FGFR3.

**Oncogenesis**
Constitutive activation of the kinase activity of FGFR3.

### t(4;12)(q11;p13) CHIC2 (BTL)/ETV6

**Note**
Rare but recurrent chromosomal abnormality. Ten cases with molecular analysis showed a CHIC2/ETV6 fusion gene.

**Disease**
AML (FAB type M0, M1, M2, therapy-related), RAEB.

**Hybrid/Mutated gene**
At least two different mechanisms.
- First mechanism: In-frame fusion between CHIC2 exons 1-3 and exons 2-8 of the ETV6 gene.
- Second mechanism: Breakpoints located in introns 1 and 2 of ETV6 but outside the CHIC2 gene, with no detectable CHIC2-ETV6 fusion gene.

**Abnormal protein**
Fusion protein contains both the HLH and ETS domains of ETV6 but no specific domain of CHIC2.
**Oncogenesis**
Ectopic expression of GSX2 detected in all cases studied, with or without the CHIC2-ETV6 fusion. GSX2 contains a homeobox domain very similar to the homeobox of the clustered HOX genes, which are involved in both normal and abnormal hematopoiesis. Overexpression of GSX2, but not CHIC2-ETV6 has transforming properties.

**t(5;12)(q31;p13) ACSL6/ETV6**

**Note**
Recurrent translocation occurring in various myeloid malignancies, often associated with eosinophilia. Only seven cases with molecular analysis.

**Disease**
Myelodysplastic syndrome (RAEB), AML, AEL, atypical CML, Polycythemia Vera.

**Hybrid/Mutated gene**
Different fusion genes are generated in four patients:
- in-frame fusion of exon 1 of ETV6 to the 3'UTR of ACSL6
- out-of-frame fusion of exon 2 of ETV6 to exon 11 of ACSL6
- out-of-frame fusion of exon 1 of ETV6 to exon 1 of ACSL6
- in-frame fusion of exon 1 of ETV6 to almost the complete ACSL6 (breakpoint at the 5' end of the ACSL6 gene).

**Oncogenesis**
Given the absence of a common in-frame fusion gene generated by the t(5;12)(q31;p13) and the heterogeneity in the localization of the ACSL6 breakpoints, no common fusion protein can explain the pathogenic character of the translocation. IL3, located near the breakpoint at 5q31, is ectopically expressed in the leukemic cells, leading to a proliferative defect.

**t(5;12)(q33;p13) PDGFRB/ETV6**

**Note**
Recurrent chromosomal abnormality (dozens of cases).

**Disease**
Myeloproliferative/myelodysplastic syndrome (RAEB), AML, AEL, atypical Philadelphia-negative CML, and CMML with eosinophilia.

**Hybrid/Mutated gene**
Exon 4 of the ETV6 gene is generally fused in-frame to exon 11 of the PDGFRB gene. Two cases showing a different fusion gene with the ETV6 breakpoint in intron 7.

**Abnormal protein**
Protein includes the HLH domain of ETV6 and the tyrosine kinase domain of PDGFRB.

In both cases showing a different fusion gene, the fusion protein retains the internal domain of ETV6 which has the ability to bind to corepressors and induce the transcription-repressive activity of ETV6.

**Oncogenesis**
Constitutive activation of the kinase activity of PDGFRB.
In both cases showing a different fusion gene, it is likely that the fusion protein acts differently from that observed in the other cases.

**t(6;12)(q23;p13) STL/ETV6**

**Note**
Only one case reported.

**Disease**
B-cell ALL.

**Hybrid/Mutated gene**
Fusion gene only retains the first two exons of ETV6.

**Abnormal protein**
Fusion protein contains no important domains (HLH or ETS) of ETV6.

**Oncogenesis**
It is likely that the truncated ETV6 contributes to leukemogenesis through ETV6 haploinsufficiency.

**t(7;12)(q36;p13) MNX1(HLXB9)/ETV6**

**Note**
Recurrent translocation found in 20 to 30% of AML children less than 18 months of age. The 7q36 breakpoint heterogeneity suggests that this translocation does not lead to the formation of a unique fusion gene. Six cases with molecular analysis showing a MNX1/ETV6 fusion.

**Disease**
AML

**Hybrid/Mutated gene**
5' MNX1-3' ETV6 resulting in a transcript in which MNX1 exon 1 is joined with ETV6 exon 3.

**Abnormal protein**
Protein contains the HLH and ETS domains of ETV6 but not the homeobox domain of MNX1.

**Oncogenesis**
It is thought that the chimeric protein acts as an aberrant transcription factor, which could affect both MNX1 and ETV6 pathways of transcription modulation.

**dic(9;12)(p13;p13) PAX5/ETV6**

**Note**
Nonrandom chromosome abnormality found in about 1% of childhood B-cell ALL.

**Disease**
ALL.
Hybrid/Mutated gene
5'PAX5-3'ETV6 transcript with fusion of exon 4 of PAX5 to exon 3 of ETV6.
Abnormal protein
The PAX5-ETV6 protein contains the "paired box" (DNA binding) domain of PAX5 fused to the HLH and ETS-binding domains of ETV6.
Oncogenesis
It is thought that the chimeric protein could act as an aberrant transcription factor, which could affect both PAX5 and ETV6 pathways of transcription modulation.

`t(9;12) (p24;p13) JAK2/ETV6`
Note
Only six cases described with ETV6/JAK2 fusion.
Disease
Pre-B ALL, atypical CML, T-cell ALL.
Hybrid/Mutated gene
Breakpoint variability: introns 4 and 5 of ETV6 and introns 12 and 17 of JAK2.
Abnormal protein
Protein retains the HLH domain of ETV6 but different domains of JAK2 (complete JH2 and JH1 in one case, only part of JH2 in the other).
Oncogenesis
Constitutive activation of the kinase activity of JAK2.

`t(9;12)(q22;p13) SYK/ETV6`
Note
Only two cases reported.
Disease
MDS.
Hybrid/Mutated gene
Chimeric gene fuses the first 5 exons of ETV6 with SYK starting with exon 5.
Abnormal protein
Fusion protein contains the HLH domain of ETV6 with part of the C-terminal SH2 and the complete protein kinase domain of SYK.
Oncogenesis
Constitutive activation of the SYK kinase activity.

`t(9;12)(q34;p13) ABL1/ETV6`
Note
26 cases described with different hemopathies but eosinophilia is a common feature.
Disease
Acute myeloblastic leukemia (AML), chronic myelogenous leukemia (CML), B-cell acute lymphocytic leukemia (ALL), T-cell ALL, MDS (RAEB), chronic myeloproliferative neoplasm, Philadelphia chromosome-negative CML.
Hybrid/Mutated gene
Two ETV6-ABL1 transcripts are usually identified: one joining exon 5 of ETV6 to exon 2 of ABL1 and one joining ETV6 exon 4 to ABL1 exon 2.
Abnormal protein
The protein retains all three SH domains, including the tyrosine kinase domain, of ABL1 and the HLH domain of ETV6.
Oncogenesis
Tyrosine kinase activation of ABL1.

`t(10;12)(q24;p13) GOT1/ETV6`
Note
Two cases of MDS described.
Disease
MDS (RA and RAE).
Hybrid/Mutated gene
Transcript containing exon 2 to exon 9 of GOT1 and the first 2 or 3 exons of ETV6.
Abnormal protein
Absence or truncation of the HLH domain of ETV6 in the protein.
Oncogenesis
Possibly inactivation of the wild type ETV6.

`t(12;13)(p13;q12) ETV6/CDX2`
Note
The t(12;13)(p13;q12-14) is a rare, but recurrent, translocation reported in a range of malignant hemopathies. However, it is evident from FISH studies that they are heterogeneous at the molecular level.
Disease
CML in transformation, myelodysplastic syndrome (MDS), acute non lymphocytic leukemia (ANLL), B and T- ALL. A sole case with ETV6/CDX2 fusion in a AML-M1.
Hybrid/Mutated gene
One in-frame fusion between exon 2 of ETV6 and exon 2 of CDX2; one fusion introducing an in-frame stop codon.
Abnormal protein
ETV6 contributes no functional domain to the fusion protein.
Oncogenesis
It is likely that the ETV6 promoter drives the transcription and ectopic activation of CDX2, which has leukemogenesis properties.

`t(12;13)(p13;q14) ETV6/TTL [not annotated gene in HGNC]`
Note
Identified in several cases of ALL and less frequently in acute or chronic myeloid malignancies.
**Disease**
A sole case with TTL/ETV6 fusion in a ALL.

**Hybrid/Mutated gene**
ETV6/TTL fusion transcript: 3’ TTL sequence introduces an in-frame stop codon after the end of ETV6 exon 1.
TTL-ETV6 transcript is a direct in-frame fusion between TTL exon 5 and ETV6 exon 2.

**Abnormal protein**
No ETV6 functional domains in the ETV6/TTL protein; HLH and ETS domains conserved in TTL-ETV6 protein.

**Oncogenesis**
Chimeric protein could act as an aberrant transcription factor, affecting the ETV6 pathway of transcription modulation, or there could be a loss of function of ETV6 and/or TTL.

**t(12;15)(p13;q25) ETV6/NTRK3**

**Disease**
Congenital Fibrosarcoma, Congenital Mesoblastic Nephroma (cellular and mixed variants), Secretory Ductal Carcinoma of Breast, rarely in AML (M0, M2) and chronic eosinophilia leukemia (1 case).

**Hybrid/Mutated gene**
5’ ETV6-3’ NTRK3.

**Abnormal protein**
Fusion protein retains the HLH domain of ETV6 and the protein tyrosine kinase (PTK) domain of NTRK3.

**Oncogenesis**
 Constitutive active tyrosine kinase.

**t(12;17)(p13;p13) ETV6/PER1**

**Disease**
Only one case of AML evolving from CMML.

**Hybrid/Mutated gene**
Fusion between exon 1 of the ETV6 gene and exon 22 and part of intron 21 of PER1.

**Abnormal protein**
No protein as PER1 has an antisense orientation.

**Oncogenesis**
It is proposed that PER1 inactivation or deregulated expression of genes located close to the breakpoint, such as HES7 or STK12, could contribute to leukemogenesis.

**t(12;21)(p13;q22) ETV6/RUNX1**

**Note**
Most common structural chromosomal abnormality in pediatric common or B-cell acute lymphoblastic leukemia, accounting for about 20-25% of the cases.

**Disease**
Childhood B-cell (ALL).

**Hybrid/Mutated gene**
Fusion of the 5’ region of ETV6 (from exon 1 to 5) with almost the entire coding region of RUNX1.

**Abnormal protein**
The fusion protein retains the HLH domain and the central repression domain of ETV6 as well as the RHD (Runt homology domain) and the transcription activation domain of RUNX1.

**Oncogenesis**
The ETV6-RUNX1 fusion protein retains the ability to bind the RUNX1 target sequences and functions as a histone deacetylase (HDAC)-dependent repressor, causing deregulation of the RUNX1 target genes. ETV6-RUNX1 is also likely to disrupt normal ETV6 functions through HLH-mediated heterodimerization.

**t(12;22)(p13;q11) MN1/ETV6**

**Note**
Rare anomaly in myeloid hemopathies (6 cases with molecular analysis).

**Disease**
Myeloproliferative disorder, myelodysplastic syndrome, AML.

**Hybrid/Mutated gene**
Two different types of MN1/ETV6 fusion (types I and II).

**Abnormal protein**
Type I fusion protein contains almost the entire MN1 fused to ETV6 at a position N terminal to the HLH domain whereas type II fusion protein has only part of the HLH domain, making it nonfunctional. Both fusion types retain the ETS domain.

**Oncogenesis**
Could act as an altered transcription factor by activating ETV6-responsive transcription and/or inhibiting RAR-mediated transcription and/or being a dominant-negative suppressor of MN1.

**t(4;12)(q23;p13) ETV6/PDGFRA**

**Note**
PDGFRA is a gene found to be fused with several partners in chronic eosinophilic leukemia.

**Disease**
Only one case of myeloproliferative neoplasm associated with hypereosinophilia and ETV6/PDGFRA fusion.

**Hybrid/Mutated gene**
In-frame fusion gene between ETV6 exon 6 and PDGFRA exon 11.

**Abnormal protein**
Protein retains most of ETV6, including the HLH domain and part of the ETS domain, fused to the WW-like domain and the kinase domain of PDGFRA.
Oncogenesis
Constitutive protein kinase activation.

\textbf{t(12;13)(p13;q12) ETV6/FLT3}

\textbf{Note}
FLT3 is one of the most frequently mutated genes in hematological malignancies, being found in about 30% of AML patients and rarely in ALL patients.

\textbf{Disease}
Three cases of myeloproliferative neoplasm with hypereosinophilia associated with ETV6/FLT3 fusion.

\textbf{Hybrid/Mutated gene}
In-frame fusion gene between ETV6 exon 4 or exon 5 and FLT3 exon 14.

\textbf{Abnormal protein}
Protein retains the HLH domain of ETV6 and the tyrosine kinase domains of FLT3.

\textbf{Oncogenesis}
Constitutively tyrosine kinase activation.

\textbf{t(6;12)(q21;p13) ETV6/FRK}

\textbf{Disease}
Only one case of AML-M4.

\textbf{Hybrid/Mutated gene}
ETV6 exon 4 fused in frame to exon 3 of FRK.

\textbf{Abnormal protein}
Chimeric protein composed of the HLH domain of ETV6 and most of the SH2 (likely to be nonfunctional) and the kinase domains of FRK.

\textbf{Oncogenesis}
Dual action of constitutively tyrosine kinase activation and dominant-negative effect on ETV6-mediated transcriptional repression.

\textbf{ins(12;8)(p13;q11q21) ETV6/LYN}

\textbf{Disease}
Only one case of primary myelofibrosis associated with ETV6/LYN.

\textbf{Hybrid/Mutated gene}
Chimeric gene consists of the 5’ region of ETV6 (breakpoint in intron 5) and the 3’ region of LYN.

\textbf{Abnormal protein}
Chimeric protein composed of the HLH domain of ETV6 and the tyrosine kinase domain of LYN.

\textbf{Oncogenesis}
Dual action of constitutively tyrosine kinase activation and dominant-negative effect on ETV6-mediated transcriptional repression.

\textbf{inv(12)(p13q13) ETV6/PTPRR}

\textbf{Disease}
Only one case of AML-M2 associated with ETV6/PTPRR.

\textbf{Hybrid/Mutated gene}
ETV6 exon 4 is fused to exon 7 of the PTPRR; 10 isoforms through alternative splicing.

\textbf{Abnormal protein}
A truncated ETV6 including the HLH domain but no functional domains of PTPRR due to frameshift and a chimeric ETV6-PTPRR protein that includes the HLH domain of ETV6 and the protein tyrosine phosphatase domain of PTPRR among others.

\textbf{Oncogenesis}
Dominant negative effect over transcriptional repression mediated by wild-type ETV6.

\textbf{t(8;12)(q13;p13) ETV6/NCOA2}

\textbf{Disease}
Pediatric acute biphenotypic leukemia (6 cases with ETV6/NCOA2), adult T-ALL (1 case).

\textbf{Hybrid/Mutated gene}
Two different in-frame fusions are known:
- one between ETV6 exon 4 and NCOA2 exon 15 (five cases)
- one between ETV6 exon 5 and NCOA2 exon 14 (one case).

\textbf{Abnormal protein}
Protein consists of the HLH domain of ETV6 and the CBP interaction and the AD2 (transactivation domain 2) acetyltransferase domains of NCOA2.

\textbf{Oncogenesis}
It is hypothesized that the ETV6-NCOA2 protein acts as a modulator of the transcriptional activity of CBP-dependent activators or can recruit CBP to ETV6 target genes resulting in their constitutive activation.

\textbf{Cryptic rearrangement shown by FISH between 12p13 and 12q24 ETV6/BAZ2A in a t(?1;12)(q42;p13)}

\textbf{Disease}
Only one case of pre-B ALL.

\textbf{Hybrid/Mutated gene}
Fusion of ETV6 and BAZ2A consisting of exons 1 and 2 of ETV6 and a sequence from intron 1 of BAZ2A.

\textbf{Abnormal protein}
No chimeric protein expected to be produced, but, maybe, a truncated ETV6.

\textbf{Oncogenesis}
It is likely that the truncated ETV6 contributes to leukemogenesis through ETV6 haploinsufficiency.

\textbf{t(5;12;22)(q13;p13;q11) ETV6/FCHO2}

\textbf{Disease}
Only one case of AML-M1.

\textbf{Hybrid/Mutated gene}
Transcript consisting of ETV6 exons 1 and 2 with sequences of FCHO2.
Abnormal protein
No chimeric protein expected to be produced (genes in opposite orientations following the translocation), but, maybe, a truncated ETV6.

Oncogenesis
It is likely that the truncated ETV6 contributes to leukemogenesis through ETV6 haploinsufficiency.

t(12;14)(p13;q32) ETV6/IGH co-localization

Disease
One case of pediatric pre-B ALL.

Cytogenetics
Co-localization of both ETV6 and IGH signals by FISH (no molecular analysis).

t(12;22)(p13;q12) ETV6/EMID1

Disease
Only one case of AML-M2.

Cytogenetics
Co-localization of both ETV6 and EMID1 signals by FISH (no molecular analysis).

Abnormal protein
No chimeric protein expected to be produced (genes in opposite orientations following the translocation), but, maybe, a truncated ETV6.

t(12;17)(p13;q21) ETV6/RARA

Disease
Only one case of myelofibrosis evolved in AML.

Cytogenetics
Co-localization of both ETV6 and RARA signals by FISH (no molecular analysis).

Abnormal protein
No chimeric protein expected to be produced (genes in opposite orientations following the translocation), but, maybe, a truncated ETV6.

t(5;12)(p13;p13) NIPBL/ETV6

Disease
Only one case of acute megakaryoblastic leukemia (AML-M7).

Cytogenetics
Co-localization of both ETV6 and NIPBL signals by FISH (no molecular analysis).

Breakpoints
See figures below.

References


ETV6 (ets variant 6)


This article should be referenced as such:

Gene Section

Review

EZH2 (enhancer of zeste homolog 2 (Drosophila))

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Abstract

Review on EZH2, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: ENX-1, ENX1, EZH1, EZH2b, KMT6, KMT6A, WVS, WVS2
HGNC (Hugo): EZH2
Location: 7q36.1
Local order
Based on MapViewer, gene flanking EZH2 oriented on 7q35-q36 are:
- CUL1 (cullin 1); 7q36.1
- RNU7-20P (RNA, U7 small nuclear 20 pseudogene); 7q36.1
- EZH2; 7q35-q36.

DNA/RNA

Description

The EZH2 gene is located on chromosome 7, starting from 148504464 and ends at 148581441 bp. This gene encodes a member of the Polycomb-group (PcG) family. PcG family members form multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes.

Transcription

Multiple alternatively spliced transcript variants have been identified for this gene. These include 5 histone-lysine N-methyltransferase EZH2 isoforms (-a/-b/-c/-d/-e). The first variant (a) has the longest isoform of histone-lysine N-methyltransferase EZH2. The second variant (b) does not have an in-frame exon and an in-frame segment in the coding region, while (c) and (d) variants lack an in-frame segment in the coding region and two in-frame segments in the coding region, respectively, as compared to (a) variant. The last variant (e) has an alternate 5’ UTR exon and lacks an in-frame exon and two in-frame segments in the coding region, as compared to (a) variant.

Figure 1. Location of EZH2 in chromosome 7, q35-36, which is located within 148504464 and 148581441 bp.
**Protein**

**Description**
EZH2 protein is the catalytic subunit of Polycomb Repressive Complex 2, one of the two-multimeric repressive complexes in the organization of the PcG.

**Function**
PcG proteins act as an important epigenetic mediator that can repress gene expression by forming multiple complexes leading to trimethylation at lysine 27 of histone H3 (H3K27me3; Cao et al., 2002; Viré et al., 2006). On the one hand, EZH2 is a histone methyltransferase, which plays an important role in tumor development (Santos-Rosa and Caldas, 2005). Moreover, this protein might also play essential roles in the control of central nervous systems by regulating the dopamine receptor D4 (Unland et al., 2014).

**Homology**
The EZH2 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, fruit fly, and mosquito.

**Mutations**

**Note**
Several mutations have been reported in EZH2 gene, which has been shown to be associated with different human diseases (e.g., Weaver syndrome, lymphoma and myeloid neoplasms). In particular, Morin and collaborators, found that the mutation of EZH2 Y641, within the SET domain, is correlated with poor prognosis in myeloid neoplasms. They observed various heterozygous mutations at Y641 in 7% of follicular lymphomas and 22% of diffuse large cell B-cell lymphomas of germinal center origin (Morin et al., 2010), which increased the level of H3K27me3 (Chase and Cross, 2011). Furthermore EZH2 mutations have not yet been reported in several other human diseases such as pancreatic ductal adenocarcinoma, but we cannot exclude that such somatic alterations might occur. Moreover, more than 4941 single nucleotide variations (SNPs) have been reported in the EZH2 gene (31th of January 2014, dbSNP), such as rs193921147, rs193921148, rs397515547, rs397515548, rs734004, rs12670401, rs6950683 etc.

**Implicated in Human diseases**

**Note**
EZH2 is a histone methyltransferase, which is involved in the regulation of cell fate, and maintaining the balance between self-renewal and differentiation (Chang et al., 2011; Cao et al., 2002; Lund et al., 2014). This protein acts as an epigenetic mediator that can suppress gene expression by histones methylation at H3k27 (Cao et al., 2002; Viré et al., 2006). EZH2 is up-regulated in many tumors, such as breast and prostate cancer, which has been shown to be associated with tumor growth, invasion, and...
metastasis as well as poor prognosis (Santos-Rosa and Caldas, 2005; Chang and Hung, 2012).

**Pancreatic cancer**

**Note**

EZH2 is found to be overexpressed in a variety of carcinomas including pancreatic adenocarcinoma (PAC), and has been shown to be associated with decreased E-cadherin expression and poor prognosis in PAC patients (Toll et al., 2010). In particular, Toll and collaborators, evaluated the correlation of EZH2 with E-cadherin expression in 54 pancreatic adenocarcinomas, 13 intraductal papillary mucinous neoplasms (IPMN), and 6 chronic pancreatitis cases, and assessed response to gemcitabine in relation to EZH2 expression in tumor cells. This study showed that high EZH2 expression in pancreatic adenocarcinoma was significantly associated with decreased E-cadherin expression and more aggressive disease. Moreover, they also observed high EZH2 expression in IPMN tissue with moderate to severe dysplasia, but not in chronic pancreatitis.

In the study by Ougolkov and colleagues, EZH2 was identified as an important factor in pancreatic ductal adenocarcinoma (PDAC) cell chemoresistance. In particular, they showed that EZH2 depletion by RNA-interference sensitized PDAC cells to gemcitabine (Ougolkov et al., 2008). Furthermore, in our recent study, we showed that inhibition of EZH2 by EZH2 inhibitor DZNep synergistically increased the antiproliferative activity of gemcitabine (first line agent in treatment of PDAC) through inhibition of cell proliferation and migration, and increasing apoptosis (Avan et al., 2012).

**Chronic pancreatitis**

**Note**

Mallen-St Clair and colleagues published an elegant study illustrating that the EZH2 connects pancreatitis to acinar cell regeneration, by providing a mechanism of protection against progression to cancerous lesions (Mallen-St Clair et al., 2012). In this study they showed that EZH2 is overexpressed in patients suffering from chronic pancreatitis. In particular, their findings revealed that EZH2 is constraining neoplastic progression through homeostatic mechanisms that control pancreatic regeneration (Mallen-St Clair et al., 2012).

**Prostate cancer**

**Note**

Varambally and collaborators, in 2002, demonstrated that EZH2 is up-regulated in hormone-refractory, metastatic prostate cancer. They found that small interfering RNA against EZH2 reduced the EZH2 protein expression in prostate cells and inhibited cell proliferation in vitro, while ectopic expression of EZH2 in prostate cells induces transcriptional repression of a specific cohort of genes. They also showed that EZH2 up-regulation was significantly associated with the progression of prostate cancer and poor clinical outcome (Varambally et al., 2002). Moreover, deletions of microRNA-101 in prostate cancer resulted as a negative regulator of EZH2 expression, providing a possible mechanism for EZH2 overexpression (Cao et al., 2010).

**Bladder carcinoma**

**Note**

Kleer and collaborators explored the functional role of EZH2 in cancer cell invasion and breast cancer progression, and evaluated the expression of EZH2 in 280 patients. They showed that EZH2 transcript and protein were consistently elevated in invasive breast carcinoma compared to normal breast epithelia. Moreover, tissue microarray analysis illustrated that the levels of EZH2 expression were strongly associated with breast cancer aggressiveness. In particular, EZH2 overexpression in immortalized human mammary epithelial cell lines stimulated anchorage-independent growth and cell invasion in the cells. In this study they identified EZH2 as a marker of aggressive breast cancer, which promotes neoplastic transformation of breast epithelial cells (Kleer et al., 2003).

**Gastric cancer**

**Note**

Several studies have been shown the role of EZH2 in bladder carcinomas (Weikert et al., 2005; Raman et al., 2005; Arisan et al., 2005). In particular, Weikert and collaborators, evaluated the EZH2 expression in 37 bladder carcinomas using real-time reverse transcription-polymerase chain reaction (RT-PCR) and correlated the data with clinicopathological findings. They found that the mRNA levels of EZH2 were significantly higher in invasive bladder carcinomas (median value, 38.92) compared to non-invasive tumors (median value, 15.51). Moreover, the level of EZH2 expression was significantly higher in grade-3, with respect to grade-1/2 lesions, suggesting its role in the progression of bladder tumors. In addition, increased EZH2 expression correlated with oncogenesis of the bladder (Arisan et al., 2005; Weikert et al., 2005).
Clinicopathological features of human gastric cancers. Immunohistochemical analysis of the tissue samples and corresponding non-cancerous gastric mucosa demonstrated that EZH2 was more highly expressed in the cancerous than in the non-cancerous tissues, and the expression levels of EZH2 were markedly associated with tumor size, depth of invasion, vessel invasion, lymph node metastasis and clinical stages. Furthermore, gastric cancer patients with high-level EZH2 expression had poorer prognosis, compared to those expressing low levels of EZH2 (Matsukawa et al., 2006).

**Lung cancer**

**Note**

Several studies have investigated the biological role and prognostic value of EZH2 in lung cancer. Recently, Xia and colleagues demonstrated that inhibition of EZH2 by RNAi enhanced irradiation-induced inhibition of human lung cancer growth in vitro and in vivo. They showed that irradiation in combination with the inhibition of EZH2 arrested A549 cells in the G1-S boundary, inhibited cell proliferation, increased the percentage of apoptotic cells in vitro, and reduced tumor size and increased survival in tumor xenograft (Xia et al., 2012). Another study evaluated the EZH2 expression in 106 patients classified as stage I non-small cell lung cancer (NSCLC). They found that patients with positive EZH2 expression had a larger tumor size and survived significantly shorter, compared to the patients with low EZH2 expression. Moreover, in vitro studies showed that knockdown of EZH2 expression in the NSCLC cell lines reduced the tumor growth rate and invasive activity, indicating that EZH2 promotes progression and invasion of NSCLC, and its expression can be considered as a novel prognostic biomarker in NSCLC (Huqun et al., 2012). Moreover, Lv and collaborators, in 2012 evaluated the expression of EZH2 in lung adenocarcinoma tissues and cell lines. They observed that EZH2 overexpression in tumor tissue significantly correlated with histological differentiation, pathological tumor-node-metastasis stage and smoking history. Moreover, overexpression of EZH2 was also detected in cisplatin-resistant cancer cells with respect to cisplatin-sensitive cells, while inhibition of EZH2 inhibited cell proliferation and migration, and induced apoptosis in both cisplatin-resistant and cisplatin-sensitive cell lines. These data suggested that EZH2 contributed to the progression of lung adenocarcinoma, and the suppression of EZH2 inhibited cell growth and sensitized cells to cisplatin in lung adenocarcinoma (Lv et al., 2012). Furthermore, Xu and collaborators, found a positive correlation between high EZH2 expression with pathologic stage, nodal involvement in lung cancer patients. In particular, they showed that overexpression of EZH2 was associated with reduced tissue inhibitor of metalloproteinase-3 expression, which was shown to be negatively associated with tumor metastasis in lung cancer (Xu et al., 2013).

**Hepatocellular carcinoma**

**Note**

Sudo and collaborators investigated the expression of EZH2 in 66 patients with hepatocellular carcinoma (HCC), using RT-PCR, and correlated its expression with clinicopathological parameters. They observed that the expression levels of EZH2 in tumor tissue specimens were significantly higher, compared to the non-tumor tissue specimens. Moreover, these analyses demonstrated that the incidence of cancer cell invasion into the portal vein was markedly increased in the group of patients with high EZH2 expression with respect to the patients with low EZH2 expression, while there was no difference in the disease-free survival rate between the two groups of patients (Sudo et al., 2005).

**Hematological malignancies**

**Note**

The role of EZH2 in hematological malignancies is still unclear. Several point mutations, resulting in gain-of-function, or inactivating mutations (loss-of-function), have been observed in lymphoma and leukemia, suggesting its role as an oncogene or tumor-suppressor gene. Visser and collaborators, evaluated the expression of both multimeric PcG protein complexes (EZH2-EED- and a BMI1-RING1-containing complex) in six cases of mantle cell lymphoma (MCL). They showed that MCL cells expressed BMI1-RING1, but not EZH2-EED, like normal mantle cells. Moreover, they showed that the up-regulation of EZH2 was associated with higher proliferation rate of haematopoietic cells (Visser et al., 2001). A recent study performed a comparative microarray analysis of gene expression in primary adult T-cell leukemia/lymphoma samples. This study found the higher levels of EZH2, RING1 and YY1 binding protein transcripts with enhanced levels of H3K27m3 in adult T-cell leukemia/lymphoma cells, compared with those in normal CD4 (+) T cells. They also showed that patients with high EZH2 expression had a significantly poorer prognosis, indicating a possible role of this gene in the oncogenesis and progression of this disease (Sasaki et al., 2011). Another gene expression profiling of Polycomb, Hox and Meis genes in 126 patients with acute myeloid leukemia showed that the expression levels of EZH2 and MEL18 were significantly higher in patients with complex karyotype and lower in CBF-mutated patients. Moreover, comparisons between the PcG and PcG-
regulated genes and clinical data demonstrated the correlations of genes involved in DNA methylation with apoptosis (BAX, Caspase 3) and multidrug-resistance (MDR1, MRP), suggesting the role of PcG and PcG-regulated genes in leukaemogenesis (Grubach et al., 2008). Moreover, Xu and collaborators examined a heterogeneous myelodysplastic syndrome (MDS)/AML population known to harbor DNA methylation of tumor-suppressor genes, such as p15INK4B. They observed that patients with p15INK4B gene methylation had a significantly higher expression of EZH2 with respect to the non-methylated counterparts, and the level of EZH2 expression correlated with poor clinical outcome (Xu et al., 2011). Conversely, Nikoloski and collaborators demonstrated the role of EZH2 as tumor suppressor gene in myelodysplastic syndromes (MDS). In this study, they sequenced the EZH2 gene in 126 patients with MDS. These analyses revealed that EZH2 gene was frequently mutated in MDS patients. Similarly, another recent study demonstrated that inhibition of EZH2 increased the tumorigenic potential and mortality of T cell acute lymphoblastic leukemia cells transplanted into NOD-SCID mice, suggesting the tumor suppressor role of PRC2 in human leukemia (Ntziachristos et al., 2012).

Qi and collaborators recently developed an EZH2-selective small-molecule inhibitor EI1 that binds to the S-adenosylmethionine of EZH2. They observed that inhibition of EZH2 by EI1 in diffused large B-cell lymphomas cells carrying the Y641 mutations decreased the cell proliferation, cell cycle arrest, and enhanced apoptosis (Qi et al., 2012). Two other recent studies have demonstrated further advances in the therapeutic potential of EZH2 inhibition to treat lymphoma. Among the compounds, which have been developed so far, EPZ005687 and GSK126 have been found to induce apoptosis in lymphoma cell lines harboring Tyr641 mutations with minimal effect on WT cells, in vitro (Knutson et al., 2012) and in vivo (McCabe et al., 2012). In particular, McCabe and collaborators, showed that GSK126 molecule inhibited tumor growth and significantly increased survival of the mice carrying lymphoma cells (McCabe et al., 2012; Lund et al., 2014). In aggregate, considering the dual function of EZH2, which has been shown to act as oncogene or tumor-suppressor gene in hematological malignancies, the therapeutic potential of EZH2 inhibitors should be evaluated carefully, to ensure achievement of beneficial effect, rather than tumorigenic effect (Lund et al., 2014).

Pediatric tumors of the central nervous system

Note
The dopamine receptor D4 (DRD4) is a G-protein-coupled receptor widely expressed throughout the central nervous system (CNS). Disruption of dopamine signaling is implyed in diseases including schizophrenia, Parkinson's and Huntington's disease (Oak et al., 2000). Recently Unland and colleagues identified DRD4 as a methylated candidate in pediatric CNS tumors, using a genome-wide methylation approach. Their analyses suggested DRD4 as a direct target of EZH2. In particular, they showed that depletio of EZH2 is sufficient to induce re-expression of DRD4, suggesting the role of EZH2 for DNA hypermethylation in the epigenetic inhibition of DRD4 (Unland et al., 2014).

Glioblastoma multiforme

Note
Overexpression of the EZH2 has been observed in different malignancies, including glioblastoma multiforme (GBM) (Venneti et al., 2013). Suvá and collaborators demonstrated that disruption of EZH2 by DZNep, or its specific down-regulation by short hairpin RNA, strongly impairs GBM cancer stem cell self-renewal in vitro and tumor-initiating capacity in vivo. They also showed the direct transcriptional regulation of c-myc by EZH2, using genome-wide expression analysis of DZNep-treated GBM, suggesting its role as a valuable new therapeutic target for management of patients with GBM (Suvá et al., 2009).

To be noted

Note
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HSPB8 (heat shock 22kDa protein 8)

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Abstract
Review on HSPB8, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity
Other names: CMT2L, DHMN2, E2IG1, H11, HMN2, HMN2A, HSP22
HGNC (Hugo): HSPB8
Location: 12q24.23

DNA/RNA

Description
HspB8 maps on chromosome 12, at 12q24.23, spanning 40.2 kb from 119611731 to 119658934. Transcription produces 5 alternatively spliced mRNAs ranging from 244 aa to 27 aa in length, only 3 of which contain an α-crystallin domain and are coding. There are 3 probable alternative promoters and 2 non-overlapping alternative last exons.

Transcription
Transcription produces 5 alternatively spliced mRNAs, which differ by truncation of the 3’ end and the presence or absence of a cassette exon. See below the features of the splice variants.

A: Accessions from pericardium, thalamus, caudate nucleus, placenta, and subthalamic nucleus. Complete mRNA, 1526 bp long, predicted protein is 244 aa, contains one α-crystallin domain.

B: Accessions from placenta cot, placenta, brain and eye. Complete mRNA, 2154 bp long, predicted protein 196 aa, contains one α-crystallin domain.

C: Accessions from placenta and placenta cot. Complete mRNA, 1795 bp long, predicted protein 130 aa, contains one α-crystallin domain.

D: Accessions from liver, spleen, head, and lung. Partial mRNA, 1337 bp, best predicted protein would have 60 aa, appears to be non-coding.

The 5 mRNA splice variants of HspB8. The empty light blue boxes represent the untranslated regions, the red boxes are exons, and the wide colored boxes are introns. Exon size is proportional to length. Introns of the same color are identical. The solid black vertical lines indicate validated cap sites at the 5’ end and the dotted black lines indicate validated polyadenylation sites at the 3’ end.
Schematic representation of the HspB8 protein, highlighting its phosphorylation sites (red arrows), naturally occurring mutations (orange type) and mutations that do not occur in nature (green). See the Mutation section below for more information on mutations.

E: Partial mRNA, 382 bp, best predicted protein would have 27 aa, appears to be non-coding.

**Pseudogene**
Unknown.

**Protein**

**Description**
HspB8 is a relatively new member of the family of mammalian small heat shock proteins (sHsps), a distinct family subset with monomer molecular masses generally in the 12-43 kDa range. HspB8 is one of 10 sHsps identified so far in the human genome (Kappé et al., 2003). It was cloned in 2000 from human melanoma and cervical cancer cells based on its homology to the protein kinase (PK) domain of the large subunit of herpes simplex virus type 2 (HSV-2) ribonucleotide reductase (R1), known as ICP10PK, and was named H11. The molecular mass of HspB8 is 22 kDa. It shares with the other sHsps, a conserved amino acid sequence, called the ‘α-crystallin domain’ that is located in the C-terminal part of the molecule. However, HspB8 also classifies as an atypical serine/threonine protein kinase (PK). Its catalytic core retains motifs I-III that are required for kinase activity. The invariant Lys residue (motif II) is at position 113 and its mutation abrogates kinase activity. Like its homologue, ICP10PK, the HspB8 autokinase activity favors Mn²⁺ ions (Smith et al., 2000).

**Physicochemical properties**
Circular dichroism spectroscopy suggests that HspB8 is an intrinsically disordered protein (IDP), meaning that it does not fold into a stable tertiary structure and has a flexible conformation. It contains many Pro residues, which enable formation of a polyproline type II (PPII) structure that contains 2-3 PXXP/PXP repeats. It is rather resistant to thermal denaturation and is very susceptible to proteolysis, consistent with theoretical predictions of disorder probability. Ultracentrifugation in glycerol gradients indicates that HspB8 is an extended monomer (Chowdary et al., 2004), a viewpoint embraced in the UniProtKB/Swiss-Prot database. This suggests that HspB8 differs from other sHsps that tend to form dimers or high-order oligomers. Additional properties that distinguish HspB8 from the other sHsps include theoretical predictions that its structure is enriched in β-strands and unordered structures and it lacks the so-called β2 strand seen in many other sHsps (reviewed in Mymrikov et al., 2011).

**Autokinase activity**
HspB8 is an atypical serine/threonine PK that resembles the HSV-2 kinase ICP10PK used in its cloning. In immunocomplex kinase assays, HspB8 undergoes autophosphorylation and it phosphorylates exogenous protein substrates. Specificity is underscored by the finding that HspB8 phosphorylates some (viz. myelin basic protein), but not other (viz. α-casein or histone IIIS) substrates. The definitive proof of intrinsic autokinase activity is provided by the loss of phosphorylation upon mutation of the invariant Lys at position 113, which is required for ATP binding (catalytic domain II). This loss of kinase activity is not due to a nonspecific conformational alteration, because it does not occur upon mutation of the adjacent Lys residue at position 115 (Smith et al., 2000; Aurelian et al., 2001; Depre et al., 2002). Further investigations performed on the isolated protein confirmed that HspB8 undergoes autophosphorylation (Chowdary et al., 2004). The rate and extent of phosphorylation are relatively low (Kim et al., 2004). However, both are significantly increased by mutations that cause physicochemical structural change, as exemplified by the significantly higher autokinase activity of the HspB8 mutant W51C, which has 7 additional β turns (Gober et al., 2003; Gober et al., 2004; Gober et al., 2005).

**Phosphorylation by other PKs**
Protein kinase C (PKC) phosphorylates HspB8 at Ser14 and Thr63, ERK1 at Ser27 and Thr87, and casein kinase 2 at a number of unidentified sites (Benndorf et al., 2001). cAMP-dependent PK phosphorylates HspB8 at Ser57. Phosphorylation of Ser57 (S57D) or Ser24 (S24D) or mutation that mimics phosphorylation at these sites affects the quaternary structure and chaperone-like activity of HspB8 (Shemetov et al., 2008). Proteomic studies have shown in vivo phosphorylation at Ser24 and...
Thr87 (Dephoure et al., 2008). However, this may be cell type and tissue specific, as phosphorylation at Tyr118 was also reported in another tissue (Rikova et al., 2007). The conditions that favor the distinct phosphorylation patterns and their effect on the structure and function of HspB8 are still unknown.

**Interactome: sHsps and other proteins and affected functions**

HspB8 interacts with most sHsps, but the stability and stoichiometry of the complexes are still unknown. All methods revealed tight interaction with HspB7, but cross-linking and immunoprecipitation failed to reveal a tight interaction with HspB1 (Hsp27). Interaction with HspB1 and HspB6 is affected by their mutation at sites that mimic phosphorylation (Ser15 and Ser16, respectively) (Sun et al., 2006). HspB8 mutation at position 51 interferes with its ability to interact with HspB1 (Smith et al., 2000). The functions regulated by HspB8 interaction with the sHsps are largely unknown, but interaction with HspB1 appears to affect NK activity (reviewed in: Hu et al., 2007; Mymrikov et al., 2011; Arrigo, 2013).

HspB8 also interacts with other proteins. HspB8 residues 62-133 interact with the RNA-binding protein Sam68 that is involved in transportation and processing of RNA (Badri et al., 2006). Because Sam68 interacts with Src-kinase at an overlapping site, its interaction with HspB8 during mitosis may indirectly regulate the intracellular localization and/or activity of Src-kinase, thereby affecting gene expression (transcription/translation). Ribonucleoprotein processing is likely affected by the interaction between HspB8 and Ddx20 (gemin3, Dp120), a protein that has ATP-dependent RNA unwinding (helicase) activity and is involved in spliceosome assembly and RNA processing (Sun et al., 2010). HspB8 also interacts with Destrin (DSTN) a cytoskeleton structural and fibrillar protein, thereby affecting actin depolymerization. It inhibits Rho GTPase and thereby functions in tachycardia remodeling, providing a protective function. It is suggested that the unique ability of HspB8 to inhibit stress fiber formation may be connected with its function in autophagy activation, which in turn acts as a trigger in RhoA pathway initiation (Ke et al., 2011). HspB8 interacts with aggregation-associated proteins, such as α synuclein, SOD1, TDP-43 and PolyQ, thereby inhibiting aggregation or fostering aggregate degradation. It is also a Toll-like receptor-4 (TLR-4) ligand causing dendritic cell activation and immunomodulation and it interacts with the cytokine-induced apoptosis inhibitor CIAPIN1, but the resulting functional modulation is still unclear (reviewed in: Arrigo, 2013). Finally, HspB8 binds Akt and 5'-AMP-activated PK, thereby promoting their nuclear translocation and cell survival (Depre et al., 2006), and it interacts with Bag-3 to regulate autophagy and with eukaryotic initiation factor 2 (eIF2) to inhibit translation (Carra, 2009).

**Expression**

**Expression in human tissues**

HspB8 is predominantly expressed in human skeletal and smooth muscle, heart, and brain. Lower expression levels are seen in prostate, placenta, lung, kidney, and skin and there is no expression in ovaries, testes, liver, pancreas, and spleen (Yu et al., 2001). Its expression may be altered in tumor as compared to normal tissues (Gober et al., 2003). In human skin, HspB8 is expressed in basal keratinocytes with long-term in vitro growth potential, which are considered the epidermis stem cells, and it is required for their proliferation (Aurelian et al., 2001).

**Stress-induced expression**

HspB8 has two heat-shock transcription factor-1 (HSF-1) binding sites, 1000 bases upstream of the translation initiation site. However, its expression is not always heat inducible and it can be upregulated by diverse stress conditions. For example, HspB8 expression is not heat-inducible in melanocytes (Smith et al., 2011) and it is upregulated by sublethal sodium arsenite and oxidative and hyperosmotic stress in neurons, where it likely contributes protective activity (Bartelt-Kirbach and Golenhofen, 2014).

**DNA methylation and the regulation of expression/function**

HspB8 differs from other Hsps in that its expression in human cells is subject to methylation-associated repression. It has a CpG island at the 5'UTR, 216 bp upstream of the transcription start site and is silenced by aberrant DNA methylation in some tumors, notably melanoma, prostate cancer, Ewing's sarcoma and hematologic malignancies (viz. leukemia, lymphoma). In these tissues/cells, restored HspB8 expression has anti-proliferative and pro-apoptotic activity (Smith et al., 2000; Gober et al., 2003; Li et al., 2007; Cui et al., 2012). However, HspB8 is overexpressed in breast cancer, particularly estrogen receptor (ER)+ breast cancer and in these cells/tissues, DNA methylation contributes to the development of resistance to anti-estrogen treatment (Fan et al., 2006). High throughput cell-based screens recognized 31 kinases, including HspB8, that confer resistance to tamoxifen therapy. They identified HspB8 as the expression signature which, by itself, predicts poor clinical outcome through inhibition of tamoxifen-induced autophagy (Gonzalez-Malerva et al., 2011). This appears to be facilitated by Lemur tyrosine kinase 3 (LMTK3), a serine/threonine kinase which functions as a regulator of the ERα and increases the levels of HspB8 (Stebbing et al., 2013). The mechanisms responsible for the cell type specificity

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*Atlas Genet Cytogenet Oncol Haematol. 2014; 18(12)*

909

**HSPB8 (heat shock 22kDa protein 8)**

**Bollino D, Aurelian L**
of the HspB8 DNA methylation patterns are still unclear. Also unclear is the relationship between methylation and accessibility to expression regulatory factors, such as HSF or estrogen (Charpentier et al., 2000; Sun et al., 2007).

**HspB and co-variant genes in differentiation/development**

HspB8 is expressed in the adult mouse and rat hippocampus, but expression is modest or absent at the embryonic and postnatal stages (Kirbach and Golenhofen, 2011). In vitro expression during the differentiation of neuronal precursor cells confirmed that HspB8 promotes neuronal, but not astrocytic differentiation and increases cell survival without affecting proliferation. Two groups of genes were found to co-vari with HspB8. In the positively correlating group, enrichment was seen for the categories "regulation of growth" (Hopx, Ddr1, Fgfr1, and Ngf) and "regulation of apoptosis" (Bag3, Fgfr1, Ngf, and Ticam1). The negatively correlating group showed enrichment for the categories "intracellular signaling" (Arhgef9, Rab14, Rap2a, Gnaq, Pclb1, Gna266, Rps22, and Usp8), "apoptosis" (Bcl2, Fnm1b, and Peg3) and "tissue morphogenesis" (Acrv1, Fnm1b, and Serpinb5). The STRING online database tool identified a cluster based around the nerve growth factor family, which contained members that positively correlate with HspB8, and another cluster that was primarily composed of apoptotic proteins which interact with the HspB8/Bag-3 complex (Ramírez-Rodriguez et al., 2013).

**Localisation**

While it is predominantly detected in the cytoplasm, HspB8 also interacts with the plasma membrane. In human neuroblastoma SK-N-SH cells, it forms tight complexes with phospholipids located in the intracellular membrane leaflet. HspB8 has two myristoylation motifs (at residues 62 and 132) and one N-glycosylation motif that likely facilitate membrane-binding and surface localization. It also co-localizes with cell surface aggregates formed by partially denatured or improperly folded proteins, for example in Alzheimer’s or Huntington disease. Unlike other Hsps, it does not always translocate to the nucleus upon heat shock stress. This is likely due to leucine-rich nuclear export signal (NES) motifs that favor cytosolic localization and are located at the N-terminus (residues 21-31) and C-terminus (residues 157-166) termini (Smith et al., 2000; Aurelian et al., 2001; Yu et al., 2001; Gober et al., 2003; Gober et al., 2004; Chowdary et al., 2007).

**Function**

**Chaperone activity**

HspB8 overexpression prevents the formation of aggregosomes containing desmin and the R120G mutant of αB-crystallin (HspB5) that correlate with the development of desmin-related cardiomyopathy and improve cardiac function (Chowdary et al., 2004; Kim et al., 2006; Sanbe et al., 2009). HspB8 also interacts with the αβ-crystallin mutants Q151X and 464delCT that form aggregates associated with the development of myofibillar myopathy (Simon et al., 2007), and amyloid β-peptides (Aβ1-42 and Aβ1-40), thereby reducing the accumulation of amyloid peptides on the cell surface and inhibiting the death of cardiovascular cells induced by Dutch-type Aβ1-40 (Wilhelmus et al., 2006). It prevents in vivo aggregation of polyglutamine containing proteins, such as a fragment of huntingtin that contains 43 Gln and the androgen receptor that contains 65 Gln residues (Wilhelmus et al., 2006; Rusmini et al., 2013) and is upregulated in neurons exposed to sublethal sodium arsenite or oxidative and hyperosmotic stress, contributing chaperone-related protection (Bartelt-Kirbach and Golenhofen, 2014). Quantitation of the in vitro chaperone-like activity of HspB8 using model substrates and size exclusion chromatography showed that HspB8 undergoes dynamic molecular transition in solution, existing in a dynamic equilibrium between various oligomers; predominantly octamers in a nonphysiological solution and mainly tetramers in a physiological solution (pH 7.4) (Yang et al., 2012). The Lys141 residue in the HspB8 α-crystallin motif is a mutational hot spot for the development of peripheral neuropathy. Two natural missense mutations, K141E and K141N, were associated with distal hereditary motor neuropathy type II (dHMN) and autosomal dominant Charcot-Marie-Tooth disease type 2L (CMT2L) in a large Chinese family (Irobi et al., 2004; Tang et al., 2005) and another mutation, K141T, was described in a Korean patient with Charcot-Marie-Tooth disease (Nakhro et al., 2013). The mutants cause neurite degeneration in motor but not sensory and cortical neurons (Irobi et al., 2010), which is apparently related to decreased chaperone-like activity measured on polyglutamine proteins as in vivo substrates (Carra et al., 2005). Ddx20 mutants fail to interact with HspB8, potentially causing different forms of inherited motor neuron diseases (Sun et al., 2010), but K141N can also cause cardiomyopathy, which is associated with the formation of perinuclear HspB8-positive aggregates that contain amyloid oligomer intermediates (Sanbe et al., 2013).

**Inhibition of unfolded protein response (UPR)**

HspB8 contributes to the proteolytic degradation of unfolded proteins, involving proteosomes or autophagy regulation. By affecting proteosome stability and intracellular localization, HspB8 induces the degradation of proteins, such as Foxo3 that prevent cardiac hypertrophy under normal
conditions (Hedhli et al., 2008). By interacting with the co-chaperone Bag-3 at the hydrophobic pocket formed by the β4- and β8-strands, it forms a complex with a 1:2 stoichiometry of Bag-3 to HspB8 (Fuchs et al., 2009) that fosters interaction with Hsc70, thereby giving rise to the multiheteromeric chaperone-assisted selective autophagy (CASA) complex. Together with the chaperone-associated ubiquitin ligase CHIP, this complex functions to remove misfolded proteins. It enables the ubiquitylation of the mutant superoxide dismutase (mSOD1) protein that is implicated in the development of amyotrophic lateral sclerosis (ALS), promoting its autophagic removal (Crippa et al., 2010a; Crippa et al., 2010b; Rosati et al., 2011; Vos et al., 2011). HspB8/Bag-3 interaction also has an important role in the protection of astrocytes against different protein aggregation diseases, apparently through autophagy-related aggregate clearance (Seidel et al., 2012) and it may contribute to chaperone-assisted selective autophagy in limb-girdle muscular dystrophy type 1D (LGMD1D), a myopathy caused by mutations of the Hsp40 family member DNAJB6 (Sato et al., 2013). During recovery from heat shock, the transcription factor nuclear factor-kappa B (NF-κB) activates selective removal of misfolded or aggregated proteins by controlling the expression of HspB8 and Bag-3 and increasing HspB8/Bag-3 complex formation, thereby increasing cell survival (Nivon et al., 2012). The CASA complex is also involved in mechanical tension, a physiological stimulus required for the development and homeostasis of locomotory, cardiovascular, respiratory, and urogenital systems and it contributes to stem cell differentiation, immune cell recruitment, and tumorigenesis. It senses the mechanical unfolding of the actin-crosslinking protein filamin, and together with CHIP, it initiates the ubiquitin-dependent autophagic sorting of damaged filamin to lysosomes for degradation (Ulbricht et al., 2013). HspB8 is also involved in spinal and bulbar muscular atrophy (SBMA), which is an X-linked neuromuscular disease characterized by the loss of motoneurons in the spinal cord and bulbar regions of the brain stem. Here, neuronal toxicity results from protein misfolding and aggregation of androgen receptor mutants that contain an elongated N-terminal poly glutamine tract (ARpolyQ) and are apparently dependent on autophagic flux failure. HspB8 restores the normal autophagic flux in motoneurons expressing ARpolyQ by exerting anti-aggregation and/or pro-degradative activity on ARpolyQ (Rusmini et al., 2013). Finally, HspB8 is upregulated in rat models of diabetes mellitus, where it is believed to play a key role in recovery and the prevention of disease-associated complications (Karthik et al., 2012; Reddy et al., 2013). However, studies of mSOD1 transgenic animals have shown that the HspB8-dependent autophagic response is much higher in muscle than spinal cord, potentially identifying a mechanism other than degradation of misfolded proteins (Crippa et al., 2013). Indeed, the HspB8/Bag-3 complex can activate phosphorylation of the α-subunit of the translation initiator factor eIF2, resulting in the general inhibition of protein synthesis and stimulating autophagy, independent of Hsc70 (Carra, 2009; Carra et al., 2009).

Signaling: stem cells, cancer and apoptosis

HspB8 has both pro- and anti-proliferative (pro-apoptotic) activity and it is cell type specific. Proliferative activity was seen in stem cells and in some cancers. For example, in human skin, HspB8 is expressed in basal keratinocytes with stem cell potential and is required for their proliferation (Aurelian et al., 2001). HspB8 is also expressed in breast cancer, glioblastoma, stomach tumors and rat pheochromocytoma (PC12) cells (Gober et al., 2005) and its expression is further increased in breast cancer cells treated with 17-β estradiol (Yang et al., 2006). In these tumors HspB8 demonstrates proliferative and anti-apoptotic properties. In breast cancer and glioblastoma cells, HspB8 functions in cell cycle regulation to prevent apoptosis, potentially through activation of the growth-associated transcription factor E2f and the cyclin-dependent kinase cdk4. HspB8 might also regulate expression of Sam68 thereby modulating the proliferative potential of the glioblastoma cells (Modem et al., 2011). Analysis of tissues from patients with breast ductal carcinoma in situ and invasive ductal carcinoma compared to normal matched controls, confirmed increased expression of HspB8 in invasive lesions, and showed that HspB8 induces anchorage independence and increases cell proliferation (Yang et al., 2006). Moreover, HspB8 overexpression was shown to increase radiation sensitivity, whereas its inhibition with siRNA was accompanied by decreased radiation sensitivity (Trent et al., 2007). In melanoma and cervical cancer, HspB8 mutation has been associated with sustained expression and the acquisition of proliferative anti-apoptotic activity as evidenced by the W51C mutant that has dominant cytoprotective activity and blocks apoptosis induced by wild type HspB8. The W51C cytoprotective activity is through activation of the B-Raf/ERK1/ERK2 survival pathway and it is associated with a 5-6-fold higher autokinase activity than that of the wild type protein (Gober et al., 2003).

By contrast, HspB8 has pro-apoptotic activity in other tumor cells. It's expression is reduced in melanoma, prostate cancer, Ewing's sarcoma and hematologic malignancies through aberrant DNA methylation. The levels of inhibition strongly
correlate with those of DNA methylation (p < .001), suggesting that HspB8 may serve as a marker for de-methylating therapeutics (Smith et al., 2011). In these tumors, restored HspB8 expression induces cell death and inhibits tumor growth in xenograft models (Gober et al., 2003; Gober et al., 2005; Li et al., 2007; Cui et al., 2012). HspB8-induced melanoma cell death is through the activation of line-specific death pathways that culminate in apoptosis and initiate with the activation of the MAP3K family member TGF-beta-activated kinase 1 (TAK1). In some of the tumor lines/xenografts, apoptosis is caused by the activation of the TAK1/p38MAPK/caspase-3/caspase-7 pathway. In others, apoptosis is through the activation of novel TAK1-dependent signaling pathways. These include (i) ASC-mediated caspase-1 activation independent of the inflammasome, (ii) Beclin-1 upregulation through mTOR phosphorylation at S2481 which is the site of intrinsic mTORC1-specific catalytic activity, and (iii) apoptosis caused by caspase-1-mediated Beclin-1 cleavage and its translocation to the mitochondria (see attached diagram below). These findings identify HspB8 as a regulator of TAK1 and mTORC1 pathways that function independent of Akt and involve inflammation-unrelated caspase-1 mediated modulation of the haploinsufficient tumor suppressor Beclin-1 (Smith et al., 2012).

**Signaling: cardiac cell hypertrophy and survival**

HspB8 expression is increased in transient ischemia, likely indicating its involvement in cell survival (Depre et al., 2001), and it has a protective role in reversible, but not irreversible myocardial injury (Depre et al., 2004). Transgenic mice with a 7-fold increase in HspB8 expression evidence significant myocardial hypertrophy accompanied by activation of Akt and p70S6 kinase (Depre et al., 2002). These mice are characterized by increased expression of glucose transporter GLUT1 in the myocytes plasma membrane, as well as increased glycogen content and phosphoglucomutase activity in the heart, suggesting that HspB8 functions in the cardiac adaptation to stress by coordinating cell growth, survival, and metabolism (Wang et al., 2004). Cell survival in this system is due to anti-apoptotic activity resulting from the direct interaction of HspB8 with Akt (Hase et al., 2005). In addition, HspB8 has metabolic and survival properties that seem to be due to direct interaction and activation of AMP-dependent protein kinase, which is responsible for increased translocation of GLUT1 to the plasma membrane and increased myocardial glycogen content (Depre et al., 2006; Danan et al., 2007). In transgenic mice with cardiac-specific HspB8 overexpression, HspB8 activates the "canonical" bone morphogenetic protein (BMP) pathway, where interaction of BMP with its receptors (BMPR-II) and Akt3 results in Smad1/Smad5/Smad8 phosphorylation. HspB8 also activates the "noncanonical" BMP pathway, promoting activation of TAK1/PI3-K/Akt and TAK1 interaction with Akt3 and BMPR-II (Sui et al., 2009). Myocardial hypertrophy results from HspB8-mediated activation of the PI3-K/Akt pathway independent of its autokinase activity (Depre et al., 2002; Sui et al., 2009). However, high dose HspB8 induces autokinase-dependent apoptosis through the inhibition of cassein kinase 2 activity (Hase et al., 2005). HspB8 upregulation by ischemia/reperfusion provides cardioprotection through enhanced mitochondrial production of nitric oxide (NO), which stimulates oxidative phosphorylation in normoxia and decreases oxidative phosphorylation and reactive oxygen species production after anoxia. The upregulation of HspB8 is correlated with increased expression of the inducible isofrom of nitric oxide synthase (Laure et al., 2012). HspB8 deletion decreases the phosphorylation of the transcription factor, STAT3, impairs transactivation of the stress response genes regulated by STAT3, and causes a significant decrease in both mitochondrial STAT3 translocation and respiration. In addition, HspB8 deletion interferes with the activation of cell survival pathways, including Akt, ERK, and iNOS (Qiu et al., 2011). In rats with induced myocardial infarction the mitochondrial translocation of HspB8 is reduced, potentially contributing to the impaired mitochondrial energy-producing ability that leads to heart failure after a myocardial infarction (Marunouchi et al., 2013).

**Tumor suppressor**

HspB8 has tumor suppressor activity. It is expressed in human melanocytes, where it functions as a cell cycle regulator and causes growth arrest through ß-catenin phosphorylation at the transcriptional activity site Ser(552) and inhibition of the cell-cycle regulatory proteins cyclin E/Cdk2 that control G1 to S transition (Smith et al., 2011). In melanoma and other cancers, its expression is inhibited through aberrant DNA methylation and restored expression through treatment with de-methylating agents causes cell death and inhibits tumor growth (Li et al., 2007; Smith et al., 2012; Cui et al., 2012). Tumor cell death is through the activation of death pathways that lead to apoptosis and activation of additional tumor suppressor functions that include the haploinsufficient tumor suppressor Beclin-1 (Smith et al., 2012). Supporting the interpretation that HspB8 has tumor suppressor function is the finding that it is highly expressed in glioblastoma cells, where its inhibition is associated with increased cell proliferation (Modem et al., 2011).
Schematic representation of the death pathways induced by HspB8 in different cells. A2058 and A375 are melanoma cells in which HspB8 signals through distinct pathways to cause cell death. While both pathways initiate with TAK1 activation, the contribution of p38MAPK and the caspases differs. In A375 cells, the TAK1/p38MAPK pathway activates caspases 3 and 7 to cause apoptosis. In A2058 cells, the TAK1/p38MAPK pathway activates caspase-3, but TAK1 also activates caspase-1 through ASC upregulation and upregulates Beclin-1 through mTOR phosphorylation at S2481. Caspase-1 cleaves Beclin-1 to promote apoptosis, but Beclin-1 also contributes to cell death through still unknown tumor suppressor functions. In both cell types, the HspB8 mutant W51C has dominant proliferative potential through its ability to trigger a B-Raf/ERK survival pathway that appears to be dependent on autokinase activity.

The cell-cycle regulatory potential of HspB8 in normal cells, its dysfunctional state in cancer cells, and its ability to induce tumor cell death, identify HspB8 as a tumor suppressor. Also characteristic of tumor suppressors, such as p53, is the cell-type specificity of the HspB8 effects and the finding that it can undergo single-site mutation to lose its activity or to acquire neoplastic potential. This is respectively exemplified by the natural mutants P173H, which is inactive, and W51C, which has proliferative (anti-apoptotic) activity (Gober et al., 2003; Smith et al., 2011; Smith et al., 2012). However, the frequency of such naturally occurring mutations is still unknown.

**Inflammation and autoimmunity**

HspB8 activates antigen-presenting dendritic cells through a TLR-4-dependent pathway and it is abundantly expressed in synovial tissues from patients with rheumatoid arthritis, potentially contributing to autoimmunity (Roelofs et al., 2006). It also induces interleukin-6 (IL-6) production in cultured pericytes and astrocytes, potentially contributing to local inflammation in Dutch type amyloidosis (Wilhelms et al., 2009). HspB8 is upregulated in synovial fibroblasts exposed to 5% cigarette smoke extract and in synovial tissues of smokers with rheumatoid arthritis (RA), suggesting that it activates signaling pathways which promote the development of autoimmunity and chronic joint inflammation (Ospelt et al., 2014). Astrocytes are key players in driving CNS inflammation. They respond to insult with a process of cellular activation known as reactive astrogliosis, a key signal of which is activated NF-κB that drives CNS inflammation (Brambilla et al., 2014). Examination of post-mortem brain tissues from patients with protein conformation disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and spinocerebellar ataxia type 3 (SCA3), revealed a strong upregulation of HspB8 and a moderate upregulation of Bag-3 in astrocytes in the cerebral areas affected by neuronal damage and degeneration. This was not the case for neurons, irrespective of their localization or the presence of protein aggregates. These findings were interpreted to suggest that the HspB8/Bag-3 complex enhances the ability of astrocytes to clear aggregated proteins released from neurons in order to maintain local tissue homeostasis and/or modulate the inflammatory response during astrogliosis (Seidel et al., 2012). The ability of HspB8 to regulate inflammatory responses is further supported by our finding that restored HspB8 expression in melanoma cells induces TAK1-dependent activation of receptor-interacting protein 2 kinase (RIP-2) that activates NF-κB and results in increased production of the pro-inflammatory cytokine TNF-α.

**Homology**

HspB8 has 32% identity and 59% homology with the HSV-2 gene ICP10PK that was used for its cloning (Gober et al., 2005). This level of sequence homology is similar to that seen for viral Bcl-2 homologues and their cellular counterparts, supporting the interpretation that the two proteins
are members of the same family. HspB8 and ICP10PK share multifunctional activities that encompass signaling, UPR, inflammatory responses, and the regulation of life-cycle potential. The contribution that ICP10PK molecular mimicry may have towards the ability of HspB8 to contribute to the development of autoimmune disorders is still unknown (Aurelian et al., 2012). However, if we accept the premise that the presence of an α-crystallin motif, even if degenerate, is a sine qua non criterion for evolutionary-based inclusion into the sHsp family, we must infer that ICP10PK is evolutionarily related to HspB8. According to this interpretation, ICP10PK is likely to have evolved from HspB8, which was originally captured by HSV-2 in order to provide survival advantages such as the inhibition of neuronal apoptosis that is required for virus growth and latency reactivation (Aurelian et al., 2012). Presumably, once it was captured and fused in-frame with the viral R1, HspB8 fell under the control of the R1 promoter, losing the regulatory constraints that define its cell-type-specific death-inducing potential while retaining kinase and ATPase-independent chaperone activity and the ability to inhibit UPR. This interpretation is supported by the recent finding of chimeric genes that consist of in-frame fused genes captured from different sources. Also consistent with this interpretation are the restriction of the homology to HSV-2, but not the closely related virus HSV-1, and the presence of missense mutations that convert HspB8 from a pro-apoptotic to a dominant anti-apoptotic protein. This interpretation is in line with current understanding of virus evolution, which recognizes viruses as "gene robbers" that have evolved after cellular species (Holmes, 2011). However, ICP10PK differs from HspB8 in that it has a transmembrane domain that is required for its kinase activity, and the possibility cannot be excluded that HspB8 evolved from ICP10PK captured by the cell from HSV-2 for an anti-stress function. Indeed, it is becoming increasingly evident that virus sequences can be incorporated into the germ-line DNA of the host, becoming inherited alongside the host sequences and contributing significant functions (Weiss and Stoye, 2013).

Mutations

Germinal

Three naturally occurring missense mutations, K141E, K141N, and K141T, located in the α-crystallin domain of HspB8 result in decreased chaperone-like activity and impaired clearance of aggregated proteins. Their expression has been implicated in the development of Charcot-Marie-Tooth disease type 2L and distal hereditary motor neuropathy (Nakrho et al., 2013).

Mutations of unknown origin: The naturally occurring mutation W51C, results in a protein with 7 additional β turns and significantly higher autokinase activity. The W51C mutation converts HspB8 from a pro-apoptotic to a dominant anti-apoptotic protein that induces cell proliferation through the B-Raf/ERK pathway independent of the cell type. In both W51C and another naturally occurring mutation, P173H, TAK-1 and its downstream pro-apoptotic pathways are not activated (Gober et al., 2003). Mutations not occurring in nature include S24D, S27D, and T87D, which interfere with the phosphorylation of HspB8. The S159D mutation has no effect on phosphorylation (Shemetov et al., 2011).

Somatic

Unknown.

Implicated in

Melanoma and other cancers

Note

HspB8 is expressed in normal melanocytes, where it causes growth arrest through β-catenin phosphorylation at the transcriptional activity site Ser (552) and inhibition of the Cyclin E/Cdk2 complex. Like the established tumor suppressors, it is silenced by aberrant DNA methylation in most melanoma tissues and in other cancers (e.g. prostate cancer, Ewing’s sarcoma, and hematologic malignancies), and its restored expression induces cell death (Gober et al., 2003; Gober et al., 2005; Li et al., 2007; Cui et al., 2012). Tumor cell death is through the activation of death pathways that lead to apoptosis as well as the activation of additional tumor suppressor functions, including upregulation of the haploinsufficient tumor suppressor Beclin-1 (Smith et al., 2012). The role of HspB8 as a tumor suppressor is further supported by the finding of a pro-tumorigenic mutation associated with increased autokinase activity (W51C). This mutation indicates that the autokinase activity is required for the HspB8 proliferative, but not anti-proliferative (pro-apoptotic) activity (Gober et al., 2003; Smith et al., 2011; Smith et al., 2012).

Charcot-Marie-Tooth disease type 2L (CMT2L)

Note

CMT is an inherited peripheral nerve disorder divided into two types: the demyelinating form (CMT1) and the axonal defective form (CMT2). Three nonsynonymous mutations of the same Lys141 residue (K141E, K141T, K141N) in HspB8 are implicated in CMT2. The lysine residue is located in the highly conserved α-crystallin domain,
and mutations in this region interfere with chaperone activity (Nakhro et al., 2013).

**Distal hereditary motor neuropathy (DHMN)**

*Note*

DHMN is a motor disorder of the peripheral nervous system that results in atrophy and muscle wasting. Two naturally occurring missense mutations, K141N and K141E, in the α-crystallin domain have been implicated in DHMN. These mutants cause neurite degeneration in motor but not sensory and cortical neurons (Irobi et al., 2010), which is apparently related to decreased chaperone-like activity measured on polyglutamine proteins as in vivo substrates (Carra et al., 2005).

**Spinal and bulbar muscular atrophy (SBMA)**

*Note*

SBMA is an X-linked neuromuscular disease characterized by the loss of motoneurons in the spinal cord and bulbar regions of the brain stem. Neuronal toxicity results from protein misfolding and aggregation of androgen receptor mutants that contain an elongated N-terminal polyglutamine tract (ARpolyQ) and is apparently dependent on autophagic flux failure. HspB8 restores the normal autophagic flux in motoneurons expressing ARpolyQ by exerting anti-aggregation and/or pro-degradative activity on ARpolyQ (Rusmini et al., 2013).

**Limb-girdle muscular dystrophy type 1D (LGMD1D)**

*Note*

LGMD1D is a form of muscular dystrophy characterized by proximal dominant muscle weakness and atrophy and caused by mutations of the Hsp40 family member DNAJB6. Immunohistochemical analysis revealed co-accumulation of HspB8 and members of the chaperone-assisted selective autophagy complex with DNAJB6 in cytoplasmic inclusions (Sato et al., 2013).

**Alzheimers**

*Note*

HspB8 is associated with the senile plaques of Alzheimer’s disease patients and the cerebral amyloid angiopathy of patients with hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D), and was shown to directly interact with amyloid-β peptide. HspB8 likely functions in maintaining the balance between production and clearance of amyloid-β, as well as its aggregation (Wilhelmus et al., 2006).

**Amyotrophic lateral sclerosis (ALS)**

*Note*

ALS is a neurodegenerative disorder characterized by the accumulation of misfolded proteins. Some familial forms of the disorder have been linked to mutations in the superoxide dismutase 1 (SOD1) gene. Mutant SOD1 proteins misfold and form aggregates, which impair proteasomal activity. HspB8 has been shown to bind and assist in the clearance of mutant SOD1 aggregates through autophagy (Crippa et al., 2010b).

**Heart failure**

*Note*

Expression of HspB8 is induced by cardiac overload. When exposed to pressure overload, mice with a HspB8 deletion experience a faster transition into heart failure and increased mortality compared to wild type controls. HspB8 deletion decreases the phosphorylation of the transcription factor, STAT3, impairs transactivation of the stress response genes regulated by STAT3, and causes a significant decrease in both mitochondrial STAT3 translocation and respiration. In addition, HspB8 deletion interferes with the activation of cell survival pathways, including Akt, ERK, and iNOS (Qiu et al., 2011).

**Diabetes**

*Note*

In a rat model of diabetes mellitus, increased levels of HspB8 have been observed in the blood plasma, where it is believed to play a key role in recovery and the prevention of disease-associated complications (Karthik et al., 2012). Upregulation of HspB8, as well as other Hsps, has also been seen in the diabetic retina, implicating it in the protection of retinal neurons and prevention of diabetic retinopathy (Reddy et al., 2013).

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Gene Section

Review

PIWIL2 (piwi-like RNA-mediated gene silencing 2)

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Abstract

Review on PIWIL2, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: CT80, HILI, PIWIL1L, mili
HGNC (Hugo): PIWIL2
Location: 8p21.3

Local order: POLR3D-LOC100507071-PIWIL2- SLC39A14-PPP3CC

Note

Human PIWIL2 or HiLi gene belongs to an evolutionarily conserved PIWI clades of Argonaute gene family that comprises four members of Argonaute genes (AGO1, AGO2, AGO3 and AGO4) and four members of PIWI genes (PIWIL1/HIWI, PIWIL2/HILI, PIWIL3 and PIWIL4/HIWI2) (Sasaki et al., 2003).

Figure 1. Diagram of Location of PIWIL2/HILI gene and its transcript variants. PIWIL2 is located in chromosome 8 at locus 8p21.3. Six transcript variants are shown.
While the PIWI family genes are exclusively expressed in the testis, the AGO family genes are ubiquitously expressed in adult tissues (Sasaki et al., 2003). All Argonaute family genes contain a central PAZ motif and a C-terminal PIWI motif (Sasaki et al., 2003). They form a RNA-induced silencing complex (RISC) to regulate a variety of biological functions through binding small RNAs (Hutvagner and Simard, 2008). PIWI genes are required for the functions in development and maintenance of germline stem cells (Cox et al., 2000).

**DNA/RNA**

**Note**

The ortholog of Drosophila PIWI gene was identified as PIWIL1/HIWI in a human testis cDNA library (Qiao et al., 2002), which is required for self-renewing and asymmetry division of germline stem cells (Lin and Spradling, 1997). PIWI2/HILI was cloned by polymerase chain reaction (PCR) of a testis cDNA library while searching databases for homologs of PIWIL1/HIWI (Sasaki et al., 2003). Normally the transcripts (mRNAs) of PIWIL2 were exclusively found expressed in testis (Sasaki et al., 2003), but it was widely dentified in various types of tumor cell lines (Lee et al., 2006; Ye et al., 2010) and stressed somatic cells with DNA damages (Yin et al., 2011b). PIWIL2/MILI was also cloned in mouse and its transcripts were only detected in the testis and spermatogonia (Wang et al., 2001).

**Description**

HILI is located in chromosome 8p21.3, starting from 22132810 and ending at 22215076 bp (ENSG00000197181), encompasses 82266 bp of DNA and consists of 23 exons (Figure 1).

**Transcription**

There are at least six transcript variants of human PIWIL2 (Figure 2). The exon 1 of variants 1 and 3 is different in size and non-coding, but they have identical open reading frame (ORF), generating identical peptides. The variant 2 has the exon 1 with the same size as variant 1 but is lack of exon 22. The variant 4 is considered to be a retained intron. The variant 5 coding Piwi-like (PL2L) proteins 60 (PL2L60) was found in the testis or tumors, which is a product of PIWIL2 alternatively activated by a putative intragenic promoter (Ye et al., 2010). The variant 6 is predicted by computer analysis and probably associated with multiple polyadenylation sites. The variant 1 (PIWIL2-001; ENST00000356766) is a canonical one, mapping to chromosome 8: 22132810-22215076 with 82.27 kb, containing 23 exons with transcript length of 5128 bps. Twenty two exons are coding one with translation length of 973 residues.

The variant 2 (PIWIL2-002; ENST00000521356) has 22 exons containing 21 coding exons, mapping to chromosome 8: 22132850-22213584 with 80.73 kb. The transcript length is 3488 bps with translation length of 937 residues.

The variant 3 (PIWIL2-003; ENST00000545409) has 23 exons with transcript length of 3442 bps, mapping to chromosome 8: 22133080-22213029 with 79.95 kb. Twenty two coding exons are translated into 973 residues, same as the variant 1. The variant 4 (PIWIL2-004; ENST00000519884) has 3 non-coding exons (intron retention), mapping to chromosome 8: 22210312-22213601 with 3.29 kb. Transcript length is 1262 bps.

The variant 5 (PIWIL2-like proteins 60 (PL2L60); AK027497) has 13 exons with transcript length of 2272 bps, mapping to chromosome 8: 22161569-22213584 with 52.015 kb. The transcript is transcribed by a predicted promoter up-stream of the exon 11, and the translation length is 530 residues (Ota et al., 2004; Ye et al., 2010).

The variant 6 [PREDICTED: Homo sapiens piwi-like RNA-mediated gene silencing 2 (PIWIL2), transcript variant X2, mRNA] has 20 exons with transcript length of 2677 bps, mapping to chromosome 8: 22132829-22179503 with 46.674 kb. The translation length is 804 residues (NCBI Reference Sequence: XM_005273551.1).

In addition to the defined transcripts listed above, there are several potentially alternatively transcribed transcripts resulted from intragenic activation of promoters of PIWIL2, such as PL2L50 and PL2L42 (Ye et al., 2010).

**Pseudogene**

No pseudogene has been found so far.

**Protein**

**Note**

HILI proteins are the products of HILI gene, belonging to the PIWI subfamily of Argonaute family proteins. The Argonaute family proteins contain two evolutionarily conserved motifs: PAZ and PIWI domains (Carmell et al., 2002). The PAZ domain is named after the proteins Piwi Argonaut and Zwille, composing of two subdomains. One subdomain is similar to the OB fold, which is well known as a single-stranded nucleic acid binding fold. The second subdomain is composed of a beta-hairpin followed by an alpha-helix. The 3’ ends of single-stranded regions of RNA binds in low-affinity in the cleft between the two subdomains. Although PAZ may not be a primary nucleic acid binding site in Dicer or RISC, it may contribute to the specific and productive incorporation of siRNAs and miRNAs into the RNAi pathway.
The PIWI domain is named after the Drosophila protein PIWI (P-element induced wimpy testes), which is essential for gametogenesis and maintenance of asymmetry division of germline stem cells (Cox et al., 1998; Cox et al., 2000; Deng and Lin, 2001; Lin and Spradling, 1997). The function of this domain is the dsRNA guided hydrolysis of ssRNA. Crystal structural analysis of Argonaute reveals that PIWI is an RNase H domain, and identifies Argonaute as Slicer, the enzyme that cleaves mRNA in the RNAi RISC complex (Song et al., 2004). The PIWI domain core has a tertiary structure belonging to the RNase H family of enzymes. By analogy to RNase H enzymes which cleave single-stranded RNA guided by the DNA strand in an RNA/DNA hybrid, the PIWI domain can be inferred to cleave single-stranded RNA, for example mRNA, guided by double stranded siRNA (Letunic et al., 2012; Schultz et al., 1998).

The Argonaute family proteins can be categorized into AGO and PIWI subfamily (Carmell et al., 2002). Both AGO and PIWI proteins can form transcriptional complexes with small RNAs to regulate gene expression. Normally AGO subfamily proteins are ubiquitously expressed in adult tissues to regulate various cell functions through binding exogenous 20-25 nucleotide (nt) small interfering RNAs (siRNAs) or endogenous 22-nt microRNAs (Carmell et al., 2002); whereas PIWI proteins are exclusively expressed in embryonic developmental stages and/or testis to regulate germline development through binding 24-31 nt piwi-interacting RNAs (piRNAs) (Lim et al., 2013b). Like all other Argonaute family members, HILI contains a central PAZ motif and a C-terminal PIWI motif (Figure 2). Aberrant or ectopic expression of HILI proteins in adult issues is likely associated with tumorigenesis (Peng and Lin, 2013; Suzuki et al., 2012).

**Description**

Six HILI isoforms of transcripts have been described (Figure 1). The HILI protein contains two characteristic domains: a PAZ domain (aa 390-524) and a PIWI domain (aa 668-956). Transcripts PIWIL2-001 and PIWIL2-003 have identical open reading frame (ORF) and encode an identical peptide of 973 amino acids (109.8 kDa). Transcript PIWIL2-002 encodes a variant of 937 residues (105.8 kDa) with a complete PAZ domain (aa 390-524) and a spliced PIWI domain (aa 668-887). PL2L-60 mRNA encodes a variant of 530 residues (59.85 kDa) with a truncated PAZ domain (aa 1-61) and a complete PIWI domain (aa 69-514). PIWIL2-004 does not encode protein product. There are at least four putative PL2L proteins including PL2L60, PL2L50, PL2L40 and PL2L40, which are the products of intragenic promoter activation of PIWIL2 and truncated at various N terminal sites of PIWIL2. Human PL2L60 has been identified and characterized (Ye et al., 2010). Argonaute proteins contain amino-terminal (N), PAZ (PIWI-ARGONAUTE-ZWILLE), MID (middle) and PIWI (P-element induced wimpy testes) domains. N domain assists the loading of small RNA and unwinding of the RNA duplex (Kwak and Tomari, 2012). PAZ domain and M domain anchors 3'-end and 5'-end of the small RNA, respectively, by providing a specific binding pocket (Jinek and Doudna, 2009). PAZ domain can bind small regulatory RNAs such as miRNAs to AGO subfamily proteins and piRNAs to PIWI subfamily proteins, whereas PIWI domain bound by mRNA contains RNase H fold, probably functioning as endonuclease to cleave the bound mRNA that is complementary to the bound small RNA (Carmell et al., 2002; Jinek and Doudna, 2009; Parker and Barford, 2006; Song et al., 2003).

**Expression**

Normally, HILI is exclusively expressed in the spermatogonia and spermatocytes of the testis (Sasaki et al., 2003) and in the female oocytes and supporting cells of human (Lim et al., 2013b). However, it can be temporarily activated in somatic cells in responding to DNA damages and in the primary cancers, and its intragenically activated products such as PL2L60 are expressed in various types of tumor cell lines (Ye et al., 2010; Yin et al., 2011b).

**Localisation**

HILI and its variants can be found in cytoplasm, nucleus or both of germline stem cells and tumor...
cells (Lee et al., 2010; Liu et al., 2010; Ye et al., 2010). They may present in chromatoïd body, a probable component of the meiotic nuage, also named P granule, a germ-cell-specific organelle required to repress transposon during meiosis (Lim et al., 2013a; Wang et al., 2009). The significance of HILI expression in cytoplasm versus in nucleus remains to be elucidated.

**Function**

PIWIL2 has multiple functions in germline development and tumorigenesis. The functions of PIWIL2 are mainly mediated by two motifs: PAZ and PIWI domains, which are highly conserved evolutionarily. PIWIL2 is associated with ribonuclease type III (DICER 1), an important component of RISC complexes (Sasaki et al., 2003).

PIWIL2 plays critical roles in the PIWI/PIWI-interacting RNA (piRNA) pathway, which is essential for spermatogenesis and transposon repression. The associated factors of the PIWI-piRNA pathway may include VASA, MAELSTROM, and TUDOR domain proteins. In coordination with the associated factors, PIWIL2 mediates piRNA biosynthesis, transcriptional silencing (Li et al., 2012), translational regulation (Unhavaithaya et al., 2009), and DNA methylation of transposons (Kuramochi-Miyagawa et al., 2008). In a mouse model, piRNA was required for de novo methylation of the differentially methylated region (DMR) of the imprinted mouse Rasgrf1 locus, but not other paternally imprinted loci, suggesting that piRNAs and a target RNA direct the sequence-specific methylation of Rasgrf1 (Watanabe et al., 2011). PIWIL2 also participate posttranslational modification through interaction with Tudor domain-containing protein TDRD1. Arginine methylation of Piwil2 proteins by PRMT5 is required for its interaction with Tdrd1 and subsequent localization to the meiotic nuage, also named P granule (Vagin et al., 2009).

In germine development, PIWIL2 may regulate the self-renewal of germline stem cells (Unhavaithaya et al., 2009) and maintain genomic integrity through interacting with piRNA to suppress the mobility of transposons, such as long interspersed nuclear elements-1 (L1, also known as LINE-1) (Marchetto et al., 2013). The piRNAs are 26 to 31 nucleotides in length and thus clearly distinct from the 21 to 23 nucleotides of microRNAs (miRNAs) or short interfering RNAs (siRNAs). PIWIL2 mediates spermatogenesis in mouse and human. DNA methylation of retrotransposons was controlled and regulated by Piwil2 partnered with piRNA (Aravin et al., 2006; Aravin et al., 2007; Kuramochi-Miyagawa et al., 2008; Xu et al., 2008).

In human, male infertility is associated with inactivation of PIWI pathway caused by the promoter hypermethylation of PIWIL2 and TDRD1. The epigenetic inactivation of PIWI gene pathway resulted in a defective production of piRNAs and a hypomethylation of the LINE-1 repetitive sequence in the affected patients (Heyn et al., 2012). In mouse testis piRNAs accumulated at the onset of meiosis (Aravin et al., 2006), silencing L1 in meiotic pachytdene cells (Di Giacomo et al., 2013). PIWIL2/MILI deficient mice were infertile (Kuramochi-Miyagawa et al., 2004). Mili-mediated secondary piRNA biogenesis fuels piRNA amplification that is absolutely required for LINE-1 silencing (De Fazio et al., 2011).

In tumor development, PIWIL2 may play multiple functions, probably depending on its activating status (Ye et al., 2010). At the beginning of tumorigenesis, PIWIL2 could temporally respond to environmental stresses, such as ionizing radiation, ultraviolet radiation and genotoxic agents, mediating chromatin relaxation to promote DNA repair and thus playing a protective role (Yin et al., 2011). PIWIL2 expression was enhanced in testicular seminomas, but not in testicular nonseminomatous tumors (Lee et al., 2006). PIWIL2 was also expressed in human and mouse tumors of various tissues (Lee et al., 2006; Ye et al., 2010).

Lee et al. showed that overexpression of PIWIL2 in a murine fibroblast cell line or human breast cancer stem cells resulted in inhibiting apoptosis and promoting proliferation and cell transformation via a signal transducer and activator of transcription 3 (STAT3)/BCLXL signaling pathway (Lee et al., 2010; Lee et al., 2006). Chen et al. demonstrated that Piwil2 transcripts was constitutively and stably expressed in murine precancerous stem cells (pCSCs) and overexpression of Piwil2 resulted in hematopoietic stem cell proliferation and transformation (Chen et al., 2007). Overexpression or ectopic expression of PIWIL2 in normal cells appeared to be associated with cell transformation and tumor initiation (Shahali et al., 2013). However, whole length of PIWIL2 was only detected in apoptotic cancer cells of primary cancers (Ye et al., 2010). PIWIL2 variants might determine the fate of a cancer cells. Ye et al. demonstrated that PIWIL2 gene could be activated via intragenic promoters, leading to expression of PIWIL2-like (PL2L) proteins, such as PL2L60, which may promote tumor survival and growth through regulating NF-κB translocation to nucleus (Ye et al., 2010). The alienation activation of PIWIL2 appeared to be associated with tumor malignancy, because PL2L proteins were mainly detected in proliferating cancer cells and cancer cell lines as well as metastatic cancer cells (Ye et al., 2010). Elucidation of the roles of PIWIL2 variants in tumorigenesis is critical for understanding complex functions of PIWIL2.
The PIWIL2 appears to be involved in various signaling transduction pathways. In TGF-β mediated signaling pathway, it suppressed TGF-β signaling pathway by physically associating with Hsp90, preventing formation of Hsp90-TβR heteromeric complexes and improving ubiquitination and degradation of TβR in a manner depending on the ubiquitin E3 ligase Smurf2 (Zhang et al., 2012). In p53 signaling pathway, the PIWIL2 repressed the tumor suppressor P53 in human cancer cells. Its PAZ domain directly associated with STAT3 protein to form a PIWIL2/STAT3/c-Src triple proteins complex, which resulted in STAT3 phosphorylation by c-Src and translocation to nucleus, then binding to P53 promoter and repressing its transcription (Lu et al., 2012). In colon cancer cell lines, the PIWIL2 could modulate matrix metalloproteinase 9 (MMP9) transcriptional activities (Li et al., 2012). In addition, silencing PIWIL2 suppressed the expression of STAT3, down-regulating Bcl-X(L) and cyclin D1, leading to a reduction of cell proliferation and survival (Lee et al., 2010). PIWIL2 may also play important roles in maintaining genomic integrity by suppressing retrotransposons, stabilizing heterochromatin structure, and regulating target genes during meiosis and mitosis. In the murine mesenchymal stem cells (MSC), Piwil2 is expressed in the cytoplasm of metaphase. In contrasting to promoting cell proliferation (Lee et al., 2010a; Lee et al., 2006; Lu et al., 2012; Zhang et al., 2012), Piwil2 did not do so in the MSCs, because knockdown of Piwil2 with a specific siRNA enhanced cell proliferation, significantly increased the number of cells in G1/S and G2/M cell cycle phases and was associated with increased expression of cell cycle genes CCND1, CDK8, microtubule regulation genes, and decreased expression of tumor suppressors Cables 1, LATS, and Cxxc4 (Wu et al., 2010).

Along with piRNAs PIWIL2 could suppress L1RNA in tumor cells. A set of piRNAs and other repeat-associated small RNAs were observed in HeLa cells. By using in situ hybridization, piR-49322 was localized in the nucleolus and around the periphery of nuclear membrane in HeLa cells. Following the overexpression of HILI, the retrotransposon element LINE1 was significantly repressed, while LINE1-associated small RNAs decreased in abundance (Wu et al., 2013).

PIWIL2 might contribute to great ape evolution. Comparative gene expression analysis of human and nonhuman primate iPSC cells revealed that levels of L1-restricting factors or DNA cytosine deaminase APOBEC3B (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3B) and PIWIL2 inversely correlated with L1 mobility and endogenous L1 mRNA levels. The increased copy numbers of species-specific L1 elements in the genome of chimpanzees compared to humans suggests that differences in L1 mobility may have differentially shaped the genomes of humans and nonhuman primates (Burns et al., 2013; Marchetto et al., 2013).

In addition, PIWIL2 was upregulated in the TGF-β induced EMT-type breast cancer stem cells (CD44+CD24-), forming a complex with piR-923 and promoting Latexin (LXN) promoter methylation (Zhang et al., 2013).

**Homology**

The PIWIL2 gene is conserved in zebrafish, mouse, rat, dog and chimpanzee.

**Mutations**

**Note**

Numerous mutations of PIWIL2/HILI have been detected in various types of primary cancers, including the cancers of breast, lung, liver, kidney, ovary, pancreas and large intestine. Copy number variation (CNV) has also been observed in the cancers of breast, large intestine, kidney, lung, ovary, pancreas, endometrium, central nervous system, hematopoietic and lymphoid, and skin (CONAN).

**Implicated in**

**Various cancers**

**Note**

Although PIWIL2 is exclusively expressed in the testis of human and animals, it has been detected in the stressing cells (Yin et al., 2011b) and almost all the various types of cancer cell lines tested of human and animals (Chen et al., 2007a; Lee et al., 2006; Ye et al., 2010) as well as in various primary cancers, including leukemia, breast tumor, medulloblastoma, rhabdomyosarcoma, colon cancer, cervical cancer and papillary thyroid carcinoma (He et al., 2010; Lee et al., 2006; Li et al., 2010; Yin et al., 2011a). Interestingly, PIWIL2 transcripts or proteins were enriched in precancerous stem cells, and cancer stem cells isolated from breast cancer and cervical cancer (Chen et al., 2007; Feng et al., 2009; Lee et al., 2010). The intragenic promoter activation resulted in alienation products of PIWIL2, such as PL2L60 protein, promoting tumor cell growth and metastasis, while full length of PIWIL2 was mainly detected in the apoptosing cancer cells of primary cancers (Ye et al., 2010). It should be noted that the failure to detect PIWIL2 transcripts in some cancers such as bladder cancers is likely associated with false negativity from RT-PCR analysis in which the primers used did not complement the alternatively transcribed mRNA (Ye et al., 2010).
**Seminoma**

**Note**
Testicular germ cell tumors can be categorized as seminoma and non-seminoma. PIWIL2 was detected in human seminoma but not in non-seminomatous tumors (Lee et al., 2006). However, a recent report showed that in addition to testicular germ cell tumor cell lines, PIWIL1, PIWIL2, PIWIL4, and TDRD1 in primary seminoma and non-seminoma testicular tumors were silenced by promoter CpG island hypermethylation. Importantly, these epigenetic lesions were associated with piRNA downregulation and loss of DNA methylation of the LINE-1 repetitive sequences (Ferreira et al., 2014).

**Bladder cancer**

**Note**
The PIWIL2 transcripts were reportedly not detected by qRT-PCR in bladder cancer cell lines and primary bladder cancers (Nikpour et al., 2009). However, a recent report showed that the PIWIL2 mRNA was detectable by qRT-PCR in 76.08% (35/46) patients with the bladder urothelial carcinoma (Cao et al., 2012). The conflicting results may be caused by the primers used, a pair of which did not complement the truncated transcripts of PIWIL2 in tumors (Ye et al., 2010).

**Cervical cancer**

**Note**
The PIWIL2 can be detected by immunohistochemical staining (IHS) in various stages of human cervical squamous cell carcinomas and adenocarcinomas. It was also detected in some metaplastic epithelial cells as well as histologically "normal" appearing tissues adjacent to malignant lesions. In Papanicolaou (Pap) test, PIWIL2 was also detected by immunocytological staining (ICS) in atypical glandular cells (AGC), low-grade (LSIL) and high-grade squamous intraepithelial lesions (HSIL). PIWIL2 is a more sensitive biomarker than p16, which was not always concomitantly detected in the same specimens (He et al., 2010). Especially, a subpopulation of cancer cells with stem-like properties expressed higher level of PIWIL2 (Feng et al., 2009).

**Colon cancer**

**Note**
The PIWIL2 and PIWIL4 were detected by immunohistochemistry (IHC) in colon cancers. The former was detected at the occurrence of colon cancers, while the later was associated with distant metastasis of the cancers (Li et al., 2010). In another report, the PIWIL2 was detected by IHC in primary colon cancer tissue and lymph node metastasis (LNM) lesions and significantly correlated with clinicopathological invasiveness, poorer five-year metastasis-free survival and poorer overall survival (Li et al., 2012; Oh et al., 2012). In addition, PIWIL2 expression was associated with poor differentiation, aggressive invasion, and perineural invasion in colorectal carcinomas (Oh et al., 2012).

**Breast cancer**

**Note**
The PIWIL2 was detected, though variable in levels, by IHS in almost all of the breast cancer samples at premalignant and malignant stages. It was detected in cytoplasm (Cyt), nucleus (N) or both cytoplasm and nucleus (C-N). The N pattern was less observed in precancerous lesions, whereas all the three patterns were observed in invasive and metastatic cancers. While the Cyt pattern correlated with ER expression; N pattern correlated with Ki67 expression. The shift of Cyt --> C-N --> N patterns were associated with the reduction of ER expression and an increase of Ki67 expressions (Liu et al., 2010). In primary breast cancers, full length of PIWIL2 was mainly expressed in apoptotosing cells while PL2L proteins, the products of PIWIL2 that was alternatively activated by intragenic promoters, appeared to be expressed in proliferating cancer cells and metastatic cancer cells (Ye et al., 2010). Like observed in cervical cancers, PIWIL2 was predominantly expressed in the breast cancer stem cells (Lee et al., 2010a; Zhang et al., 2013). Up to 90% of invasive carcinomas and 81% of carcinomas in situ expressed highest level of PIWIL2 (Lee et al., 2010).

**Thyroid cancer**

**Note**
Piwil2 proteins and mRNAs were detected by IHS and in situ hybridization (ISH) in 88.3% and 88.5% papillary thyroid carcinoma (PTC), respectively. The level of PIWIL2 expression was associated with the invasiveness and metastasis of PTC (Yin et al., 2011a).

**Gastric cancer**

**Note**
The expression of PIWIL2 detected by IHS was significantly higher in the gastric tumor tissue than that in adjacent non-tumor tissue. Expression level of PIWIL2 was positively correlated with the T stage, lymph node metastasis and clinical TNM (cTNM). Moreover, elevated PIWIL2 expression in cancer tissue predicted poorer overall survival (OS) compared with the group of lower expression (Wang et al., 2012).
**Sarcoma**

**Note**

PIWIL2 expression in 125 soft tissue sarcoma (STS) samples together with PIWIL3 and PIWIL4 expressions were measured by real-time PCR (qPCR). Low PIWIL2 or PIWIL4 mRNA expressions were significantly associated with a poor prognosis. Low expression of both genes was associated with a 2.58-fold increased risk of tumor-related death. PIWIL4 and the combined PIWIL2 and PIWIL4 mRNA levels correlated significantly with prognosis only for female but not for male patients. However, the combined low PIWIL 2 and PIWIL3 transcript levels were associated with worse survival for male patients (Greither et al., 2012).

**Prostate cancer**

**Note**

The PIWIL2 was detected using Whole Human Genome Oligo Microarrays in prostate cancer. Compared to peripheral zone, PIWIL2 was down-regulated in poorly differentiated tumors but not in moderately differentiated tumors (Shaikhibriahim et al., 2013).

**Leukemia**

**Note**

PIWIL2 expression in acute myeloid leukemia (AML) was analyzed by real-time PCR and differed in expression pattern in a gender-dependent manner (Shaikhibriahim et al., 2013).

**Ovarian cancer**

**Note**

The PIWIL2 was detected by IHS in the primary ovarian cancer and metastatic tissues from the patients with stage III epithelial ovarian cancer (EOC). Other PIWI proteins such as PIWIL1, PIWIL3, and PIWIL4 were also detected in the primary tumor and metastatic tissues (Chen et al., 2013).

**Male infertility**

**Note**

Defective PIWIL2 expression is associated with male infertility in mouse and human (Heyn et al., 2012; Kuramochi-Miyagawa et al., 2004). In human, the disorder was likely associated with the promoter hypermethylation of PIWIL2 and its associated factor TDRD1, which resulted in a defective production of piRNAs and a hypomethylation of the LINE-1 repetitive sequence in the affected patients (Heyn et al., 2012). The presence of PIWIL2 in oocytes or ovary also suggests that it may participate in similar functions during oogenesis in females (Lim et al., 2013b; Olesen et al., 2007). The link of PIWIL2/HILI single nucleotide polymorphisms (SNPs) with spermatogenic failure has not been established (Gu et al., 2010).

**To be noted**

**Note**

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Gene Section
Review

ABCC10 (ATP-binding cassette, sub-family C (CFTR/MRP), member 10)

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Abstract
Review on ABCC10, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity
Other names: EST182763, MRP7, SIMRP7
HGNC (Hugo): ABCC10
Location: 6p21.1

DNA/RNA
Note
Contains 22 exons and 21 introns (Kao et al., 2003).

Description
The gene encompasses 23065 bases.

Transcription
Transcript is 744 bps, and has 10 splice variants.

In normal tissue
A study of the ABCC10 promoter revealed that the presence of E2F and Sp1 sites are required for maximum transcription.
In addition other elements were found in the promoter including: cAMP-responsive element binding protein, estrogen receptor binding site, hepatic nuclear factor, progesterone receptor binding site, and sterol regulatory element binding protein (Dabrowska and Sirotnak, 2004).
ABCC10 transcript is widespread at low levels (Hopper et al., 2001; Maher et al., 2005) with highest expression in the pancreas. Decreased expression is observed in activated resting T and B cells (Takayanagi et al., 2004).

In cancer
In cell lines: In terms of cancer, the ABCC10 gene is expressed in human tumor cell lines, HepG2, CWR22RV1 and TSU-PR1 at greater or equal levels than ABCC1, ABCC2 and ABCC3. Novel ABCC10 transcripts have been identified in HepG2, and CWR22RV1 cells. These transcripts are heterogeneous and imply complexity in the exons and introns of this region (Dabrowska and Sirotnak, 2004).
In breast cancer: ABCC10 transcript is upregulated in breast tumors treated with 5-fluorouracil, anthracycline, cyclophosphamide or taxane based neoadjuvant chemotherapy in comparison with normal tissue. Further, ABCC10 transcript expression is associated with ER positive breast cancer (Hlavac et al., 2013).
In acute myeloid leukemia: ABCC10 transcript is also expressed in acute myeloid leukemia (Hu et al., 2011).
In hepatocellular carcinoma: Transcripts from ABCC10 and various other transporters including: ABCB6, ABCC1, ABCC4, ABCC5, and ABCC12 are upregulated in over 50% hepatocellular carcinoma in untreated patients. It was also observed that microRNA let-7a/e is able to downregulate ABCC10 transcript (Borel et al., 2012).
**Protein**

**Description**
Unglycosylated ABCC10 is ~158 kDa (Hopper et al., 2001).
ABCC10, like ABCC1, 2, 3 and 6 (see figure above A) has an N-terminal membrane spanning domain (MSD) that is not present in ABCC4, 5, 11 or 12 (figure above B). All ABCC subfamily members contain two Nucleotide Binding Domains (NBD).

**Expression**
ABCC10 has been detected in various human ocular-absorption barrier tissues including, the iris ciliary body, corneal epithelium, conjunctive epithelium, and retina (Chen et al., 2013).
ABCC10 is expressed in cell lines derived from acute myeloid leukemia (Hu et al., 2011).

**Localisation**
Localizes basolaterally in ABCC10-transfected LLC-pK1 cells (Malofeeva et al., 2012).

**Function**

**In vitro**
ABCC10 transports estrogen β glucuronide, and exhibits modest transport of leukotrienes. Similar to other subfamily members ABCC10 can efflux lipophilic anions (Chen et al., 2003).
ABCC10 is a drug efflux pump that is able to mediate transport of taxanes in vivo. In vitro studies have shown that ABCC10 confers resistance to a variety of hydrophobic drugs, including paclitaxel, docetaxel, vincristine, vinblastine, epothilone B, cytarabine, in a glutathione independent manner (Hopper-Borge et al., 2004; Hopper-Borge et al., 2009).
Prior work has shown that ABCC10 may have a role in natural killer cell mediated lysis. A report demonstrated that a peptide derived from ABCC10 binds to HLA-E and inhibits NK cell-mediated lysis in a CD94 and class I-dependent fashion (Wooden et al., 2005).
ABCC10 ATPase activity is stimulated by estradiol glucuronide, leukotriene, tamoxifen, docetaxel and ARA-C (Malofeeva et al., 2012).

**In vivo**
Abcc10 protects thymus, spleen and bone marrow when exposed to paclitaxel in vivo. Abcc10 loss promotes loss of white blood cells, increased weight loss and increased lethality in mice exposed to high doses of paclitaxel (Hopper-Borge et al., 2011).

**Homology**
ABCC10 exhibits structural and/or functional homology with other ABC transporters: CFTR, ABCC1, ABCC2, ABCC3, ABCC6. However, ABCC10 shares the lowest amino acid identity (33.8%) to ABCC1 compared to ABCC2, ABCC3, ABCC4, ABCC5 and ABCC6 (Hopper et al., 2001).
ABCC10 is present in many species including: drosophila melanogaster, xenopus, saccharomyces cerevisiae, danio rerio, sus scrofa, mus musculus, latimeria chalumnae, felis catus, bos taurus, tetranychus urticae (Schippert et al., 2008; Dermauw et al., 2013).
ABCC10 is present in all placental mammals, similar to other subfamily members, ABCC1,
ABCC3, ABCC4-7, and ABCC9 (Moitra and Dean, 2011).

**Implicated in**

**Non small cell lung cancer**

**Note**

In vitro cellular resistance in non small cell lung cancer. ABCC10 expression is a predictive biomarker for the resistance to paclitaxel in non-small cell lung cancer (Oguri et al., 2008). ABCC10/MRP7 expression is associated with vinorelbine resistance in non-small cell lung cancer (Bessho et al., 2009). Another study implicated ABCC10 in gemcitabine resistance mechanisms in non small cell carcinoma cell lines (Ikeda et al., 2011). ABCC10 is overexpressed in NSCLC. The expression of ABCC10 in adenocarcinoma is higher than in squamous cell carcinoma. ABCC10 expression in adenocarcinoma correlates with pathological grades and TNM stages (Wang et al., 2009).

**Head and neck cancer**

**Note**

Multidrug resistance protein 7 expression is involved in cross-resistance to docetaxel in salivary gland adenocarcinoma cell lines (Naramoto et al., 2007).

**Ovarian cancer**

**Note**

A ovarian carcinoma xenograft model demonstrated that intermittent docetaxel dosing of tumors promotes upregulation of MRP7 and other drug resistance genes including tubulin III, Akt2, and thioredoxin. However, when the dosing was continuous the various resistance genes did not upregulate (De Souza et al., 2011).

**Acute myeloid leukemia**

**Note**

ABCC10 may play a role in Ara-C resistance mechanisms in acute myeloid leukemia (Hu et al., 2011). ABCC10 transcript is more highly expressed in childhood AML than in many normal samples (Steinbach et al., 2006).

**Breast cancer**

**Note**

In metastatic breast cancer, a difference in progression-free survival (PFS) was found between circulating stem cells (CTCs) positive and CTCs-negative patients. PFS was shorter in patients whose CTCs expressed two or more ABC subfamilies, including ABCC10 (Gradilone et al., 2011).

ABCC10 expression may confer therapeutic resistance in solid breast tumors (submitted, Domanitskaya et al.).

**Colorectal cancer**

**Note**

An inverse correlation between ABCC10 transcript and tumor aggressiveness and tumor grade severity was found in colorectal cancer (Hlavata et al., 2012).

**Human immunodeficiency virus**

**Note**

ABCC10 polymorphisms contribute to the development of kidney tubular dysfunction (KTD) in some HIV patients (Giacomet et al., 2013). ABCC10 polymorphisms modulate blood levels of the anti HIV drugs tenofovir, and nevirapine (Pushpakom et al., 2011; Liptrott et al., 2012).

**Parkinson's disease**

**Note**


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CHST11 (carbohydrate (chondroitin 4) sulfotransferase 11)

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Abstract

Review on CHST11, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: C4ST, C4ST-1, C4ST1, HSA269537
HGNC (Hugo): CHST11
Location: 12q23.3
Local order: Centromere - NFYB - TXNRD1 - CHST11 - SLC41A2 - ALDH1L2.

DNA/RNA

Description

The CHST11 gene spans 305 kb on chromosome 12q23.3.

Transcription

The CHST11 gene contains one 5’ non-coding exon and three coding exons and is transcribed into a 5.7 kb mRNA. Transcription of CHST11 has been shown to be positively regulated by signaling through transforming growth factor-beta (TGFβ) pathways (Klüppel et al., 2002).

Genomic organization of the CHST11 locus on chromosome 12q23.3, encompassing nucleotides 104850692 to 105155792. Orientation (5’ to 3’) of CHST11, and neighbouring genes TXNRD1, SCL41A2, and ALDH1L2 are indicated by arrowheads. cen: centromere; qter: telomere of q-arm. Adapted from USCS Genome Browser, hg19 (November 2013).
Schematic illustration of the protein structure of CHST11 with known motifs indicated. CHST11 contains a transmembrane domain (TMD) for anchorage in the Golgi membrane (encoded by exon III), and a large luminal catalytic domain harboring a sulfotransferase domain, which contains a 5'-phosphosulfate site (PSB), a 3' phosphate binding site (PB), as well as four C-terminal N-glycosylation sites (N1-N4) (all encoded by exon IV).

Using a bioinformatical approach, conserved long-range cis-regulatory modules were identified in the CHST11 locus. Luciferase reporter assays identified a functional CHST11 promoter, as well as a number of cis-regulatory modules able to positively and negatively regulate CHST11 expression in a TGFβ-dependent as well as -independent manner (Willis et al., 2009).

**Protein**

**Description**

The CHST11 protein contains 352 amino acids, and has an approximate molecular mass of 43 kDa. CHST11 is a single pass type II membrane-bound protein (Klüppel, 2010). CHST11 protein contains a transmembrane domain (TMD) for anchorage in the Golgi membrane, a 5' phosphosulfate binding site (PSB), a 3' phosphate binding site (PB), required for binding of the phosphate donor PAPS and transfer of sulfate groups, and four N-linked glycosylation sites (N1 to N4) in the C-terminal end of the protein.

**Expression**

CHST11 has a highly specific temporal and spatial expression pattern during mouse embryogenesis, and has been detected in notocord, heart valves and myocardium, apical ectodermal ridge during limb generation, neural tube, hair follicles, kidney, and proliferating chondrocytes in the cartilage growth plate during skeletal development (Klüppel et al., 2002; Klüppel et al., 2005). In adult tissues, CHST11 has been reported to be widely expressed, including in spleen, thymus, bone marrow, peripheral blood leukocytes, lymph node, heart, brain, lung and placenta (Habuchi and Miyashita, 1982; Hiraoka et al., 2000; Okuda et al., 2000; Yamauchi et al., 2000).

**Localisation**

CHST11 is a single pass type II membrane-bound protein localized to the Golgi (Klüppel, 2010). However, CHST11 was initially identified as a protein secreted from chondrocytes and chondrosarcoma cells (Habuchi et al., 1991; Yamauchi et al., 1999).

**Function**

**Role in carbohydrate metabolism:**

CHST11 catalyzes the transfer of sulfate from the universal intracellular sulfate donor PAPS (3'-Phosphoadenosine 5'-phosphosulfate) to the C4 position of the glycosaminoglycan chondroitin, generating chondroitin-4-sulfate (C4S) and adenosine 3',5'-bisphosphate (Habuchi, 2000; Klüppel, 2010). Through a subsequent CHST11-independent enzymatic sulfation reaction, C4S can be transformed into the double-sulfated chondroitin sulfate-E (CS-E) (Habuchi, 2000; Klüppel, 2010). Different chondroitin sulfation forms have been shown to have distinct biological functions. CHST11 has also been shown to positively regulate chondroitin sulfate chain elongation (Anggraeni et al., 2011). N-glycosylation of CHST11 is required for its enzymatic function and heat stability (Yusa et al., 2005).

**Role in cartilage development and osteoarthritis (OA):**

Mouse CHST11 has been shown to be required for cartilage growth plate morphogenesis (Klüppel et al., 2005). Loss of CHST11 caused chondrodysplasia with severely shortened long bones, caused by shortened and thickened cartilage growth plates with disorganized and hypo-cellular cartilage growth plates with fibrillated ECM and an overall loss of chondroitin sulfate. Increased apoptosis of mutant chondrocytes was observed, and TGFβ and BMP signaling was disturbed in mutant growth plates (Klüppel et al., 2005). Many of these cartilage deficiencies are characteristic of the degenerative alterations observed in OA, a degenerative disease characterized by loss of matrix GAGs and cartilage integrity. Increased CHST11 expression has been observed in OA (Zeggini et al., 2012). Combined, these data suggest a requirement for tightly controlled regulation of CHST11 expression in the development and maintenance of healthy cartilage.

**Role in HSV infection:**

Herpes simplex virus (HSV) envelope glycoproteins utilize cell-surface GAGs to efficiently bind to and infect host cells. The gC HSV envelope protein has been suggested to bind cell-surface CS-E-proteoglycans with high affinity.
and treatment with exogenous CS-E could potently inhibit HSV infectivity, thus identifying CS-E as a negative regulator of HRAS signaling in these cells elevated CHST11 expression, thus identifying CHST11 as a negatively regulated target gene of HRAS signaling (Klüppel et al., 2012). Forced expression of CHST11 in Costello fibroblasts rescued the oncogenic HRAS signaling in these cells elevated CHST11 expression, while interference with oncogenic HRAS signaling in these cells elevated CHST11 expression, thus identifying CHST11 as a negatively regulated target gene of HRAS signaling (Klüppel et al., 2012). Forced expression of CHST11 in Costello fibroblasts rescued the proliferation and elastogenesis defects associated with oncogenic HRAS signaling in these cells (Klüppel et al., 2012). These results indicate that reduced CHST11 expression and a subsequent chondroitin sulfation imbalance mediate the effects of oncogenic HRAS signaling in the pathogenesis of Costello syndrome.

Role in cancer:
Changes in CS levels and chondroitin sulfation balance have been described during tumor progression (Picciardiello et al., 1997; Suwiwat et al., 2004; Theocharis et al., 2006; Sakko et al., 2008; Teng et al., 2008; Svensson et al., 2011; Vallen et al., 2012). Experimental elimination of chondroitin sulfate in orthotopic breast cancer mouse models lead to increased metastasis, demonstrating a critical role of chondroitin epitopes in tumor progression in vivo (Prinz et al., 2011). The CHST11 gene was highly expressed in aggressive breast cancer cells, but significantly less so in less aggressive breast cancer cell lines (Cooney et al., 2011). Moreover, a positive correlation was observed between the expression levels of CHST11 and P-selectin-mediated adherence of breast cancer cells to endothelial cells (Cooney et al., 2011). Increased expression of the CHST11 gene has been observed in multiple myeloma (Bret et al., 2009). One case report of a patient with B-cell chronic lymphocytic leukemia (B-CLL), a chromosomal translocation with breakpoints in the IGH locus on chromosome 14, and the CHST11 locus on chromosome 12 [t(12;14)(q23;q32)] was identified. The translocation breakpoint mapped to intron 2 of the CHST11 locus, and resulted in the expression of two truncated forms of CHST11 (Schmidt et al., 2004).

Role in Wnt-β-catenin signaling:
Studies were performed in mutant sog9 L-cell fibroblasts, which lack the expression of both EXT1 (Extosis-1, required for heparan sulfate biosynthesis) and CHST11 genes (Nadanaka et al., 2008). Mutant cells had a significant decrease in Wnt3a-stimulated β-catenin accumulation, which could be rescued by stably expressing CHST11, but not EXT-1 (Nadanaka et al., 2008). In addition, this study showed that the specific chondroitin sulfate form CS-E, but not the other chondroitin sulfate forms, was able to bind Wnt3a ligand with high affinity. Addition of CS-E to normal L-cells reduced β-catenin levels, much like what was seen in the sog9 mutant L-cells lacking CHST11 expression (Nadanaka et al., 2008). Together, this data suggested that the CHST11, through its ability to produce CS-E containing proteoglycans, might play a role in the Wnt/β-catenin signaling pathway. The investigators of this study suggested a model in which CHST11 expression increases the level of CS-E containing proteoglycans, which can then bind Wnt3a, and facilitate the binding of Wnt ligands to its receptor complex, thus increasing the efficiency of ligand-receptor interactions. In a follow-up study, Nadanaka et al. (2011) show that L-cells stably expressing the Wnt3a ligand had a reduction in CHST11 gene expression, and subsequently a change in sulfation balance with a higher concentration of chondroitin sulfate products with low affinity for Wnt3a ligand binding (Nadanaka et al., 2011). This allows the Wnt3a ligand to freely diffuse across L-cell fibroblast cultures. Forced expression of CHST11 was suggested to inhibit the diffusion of Wnt3a ligand in L-cell fibroblast cultures, because of the increase in production of CS-E containing proteoglycans (Nadanaka et al., 2011). These and previous studies suggested that CHST11 expression is able to inhibit Wnt3a diffusion and sustained signaling, but CHST11 gene expression is negatively regulated by active Wnt/β-catenin signaling (Nadanaka et al., 2011).
We reported the identification of the CHST11 product CS-E as an inhibitor of specific molecular and biological outcomes of Wnt3a signaling in NIH3T3 fibroblasts (Willis and Klüppel, 2012). CS-E could decrease Wnt3a signaling through negative regulation of LRP6 receptor activation. However, this inhibitory effect of CS-E only affected Wnt3a-mediated induction, but not repression, of target gene expression (Willis and Klüppel, 2012). We went on to identify a critical Wnt3a signaling threshold that differentially affects target gene induction versus repression. This Wnt3a signaling threshold also differentially controlled the effects on proliferation and serum starvation-induced apoptosis (Willis and Klüppel, 2012). These data established the feasibility to manipulate the chondroitin sulfate biosynthesis machinery, in particular CHST11, to selectively inhibit Wnt/β-catenin transcriptional programs and biological outcomes through the exploitation of intrinsic signaling thresholds (Willis and Klüppel, 2012).

**Homology**

Homologous genes: CHST12, CHST13.

**Mutations**

**Note**

A chromosomal translocation t(12;14)(q23;q32) has been described in one patient with B-cell chronic lymphocytic leukemia (B-CLL) (Schmidt et al., 2004). Breakpoints of this have been mapped to the IGH locus on chromosome 14, and the CHST11 locus on chromosome 12 [t(12;14)(q23;q32)] (Schmidt et al., 2004). The translocation breakpoint mapped to intron 2 of the CHST11 locus, and resulted in the expression of three CHST11-IgH fusion transcripts (Schmidt et al., 2004).

It was not determined whether these fusion transcripts lead to the expression of truncated CHST11 proteins, or whether the expression of the observed fusion transcripts might have any functional consequences on chondroitin sulfate biosynthesis and/or disease development or severity.

**Somatic**

This is a somatic mutation in B-CLL cells (Schmidt et al., 2004).

**Implicated in**

**B-cell chronic lymphocytic leukemia (B-CLL)**

**Cytogenetics**

Translocation t(12;14)(q23;q32).

**Hybrid/Mutated gene**

This translocation generates a IGH-CHST11 hybrid gene, with breakpoints in the IGH locus on chromosome 14, and the CHST11 locus on chromosome 12. A functional role of this hybrid...
gene in tumor progression has not been elucidated (Schmidt et al., 2004).

**Multiple myeloma**

Note

Microarray analysis identified increased expression of a number of genes involved in glycosaminoglycan biosynthesis, including CHST11 (Bret et al., 2009). The authors hypothesized that heparan sulphate and chondroitin sulphate side chains of the proteoglycan syndecan-1 play critical roles in mediating the biological changes from memory B cells to malignant plasma cells (Bret et al., 2009).

**Breast cancer**

Note

The CHST11 gene is highly expressed in aggressive breast cancer cells, but significantly less so in less aggressive breast cancer cell lines (Cooney et al., 2011). Moreover, a positive correlation was observed between the expression levels of CHST11 and P-selectin-mediated adherence of breast cancer cells to endothelial cells (Cooney et al., 2011).

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Uyama T, Ishida M, Izumikawa T, Trybala E, Tufaro F, Bergström T, Sugahara K, Kitagawa H. Chondroitin 4-O-
This article should be referenced as such:
Abstract

POU3F2, also known as BRN2, Oct7, and N-Oct3, is a member of the neural cell-specific class III POU domain transcription factors (Ryan and Rosenfeld, 1997). POU3F2-knockout causes the loss of specific neuronal lineages in the endocrine hypothalamus and the subsequent loss of the posterior pituitary gland (Nakai et al., 1995; Schonemann et al., 1995; Alvarez-Bolado et al., 1995). And also, transgenes of POU3F2 and some other few factors converted non-neural cells to neural cells in vitro (Ambasudhan et al., 2011; Lujan et al., 2012; Pang et al., 2011). These results indicate that POU3F2 is an indispensable transcription factor for neural differentiation and generation of normal nervous system, especially hypothalamus. There have been a few reports regarding the functions of POU3F2 in association with tumorigenesis. POU3F2 has been demonstrated to be an oncogene in malignant melanomas derived from the neuroectodermal cell lineage and to accelerate the growth of melanoma cells (Cook and Sturm, 2008). POU3F2 are also highly expressed in small cell lung cancers and closely associated with the cancer specific neural/neuroendocrine phenotype (Schreiber et al., 1992; Ishii et al., 2013; Sakaeda et al., 2013).

Keywords
Transcription factor, POU domain, nervous system, neural development, hypothalamus, melanoma, small cell lung cancer, neuroendocrine

Identity

Other names: BRN2, N-Oct3, OCT7, OTF-7, OTF7, POUF3, brn-2, oct-7
HGNC (Hugo): POU3F2
Location: 6q16.1

Schematic representation of POU3F2 gene and mRNA. The POU3F2 gene is located on the plus strand of 6q16.1 chromosome. It consists of one exon (4086 bp).
POU3F2 (POU class 3 homeobox 2)  

**DNA/RNA**

**Description**

POU3F2 is intron-less gene. Coding region is located in 5’ side of the gene.

**Transcription**

4108 base mRNA, coding sequence is 1332 base. There are no reports about transcriptional variant.

**Protein**

**Description**

POU3F2 belongs to the Class-III POU transcription factor family. All class-III POUs mainly express in some part of nervous system and regulate the development (Dominguez et al., 2013; Phippard et al., 1999). Two DNA binding domain, POU-specific domain, and homeodomain are conserved in POU transcription factor family.

**Expression**

POU3F2 expresses in developing nervous system and hypothalamus (Andersen and Rosenfeld, 2001). It also localizes in the developing mouse spinal cord (Tanaka et al., 2004).

**Localisation**

POU3F2 mainly localizes in nuclei.

**Function**

POU3F2 makes homodimer or heterodimer with other transcription factor and recognize specific DNA motif. POU3F2 activates the transcription of near gene. It is reported that target genes of POU3F2 are some neural genes (Blau et al., 2004), phosphodiesterase 5A (Arozarena et al., 2011), cadherin 13 (Ellmann et al., 2012), NNX2.1 (Sakaeda et al., 2013).

**Homology**

POU3F2 shows highly similarity with the other class-III POU proteins (POU3F1, POU3F3, POU3F4) especially in the DNA binding domain.

**Mutations**

**Note**

There is no report about mutation of POU3F2.

**Implicated in**

**Melanoma**

**Note**

POU3F2 is highly expressed in melanoma cells and is related to the tumorigenesis and the growth (Cook and Sturm, 2008). Suppression of POU3F2 reduced proliferation activity and tumorigenic potential of melanoma cells (Thomson et al., 1995). It is reported that high POU3F2 expression is due to the activation of MAPK signaling pathway associated with BRAF gene mutation (Goodall et al., 2004).

**Small cell lung cancer**

**Note**

Small cell lung cancer (SCLC) highly expresses POU3F2 compared with non-SCLC. POU3F2 induces several neuroendocrine specific transcription factors and marker molecules and is associated to the cell viability (Schreiber et al., 1992; Ishii et al., 2013; Sakaeda et al., 2013).

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This article should be referenced as such:
PRAME (preferentially expressed antigen in melanoma)

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Abstract
Review on PRAME, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity
Other names: CT130, MAPE, OIP-4, OIP4
HGNC (Hugo): PRAME
Location: 22q11.22

DNA/RNA
Note
PRAME is a member of a multigene family present in humans and other mammals. It was originally identified as a gene encoding a novel cancer-testis antigen that is over expressed in melanoma (Ikeda et al., 1997). Its evolution is consistent with adaptive (positive) selection similar to gene clusters involved in immunity and reproduction, such as the NALP family (Tian et al., 2009). Expression of PRAME has been shown to be regulated by hypomethylation of its promoter in AML and CML (Ortmann et al., 2008; Roman-Gomez et al., 2007).

Description
The gene is encoded on the reverse strand of chromosome 22 (22q11.22) covering a region of approximately 12 kilobases and is within the human immunoglobulin lambda gene locus (Kawasaki et al., 1997). This locus contains a large number of VI gene segments which code for production of I light chains during B cell development and several other non-immunoglobulin genes, for example, tandem Suppressor of Hairy Wing genes (SUHW1/ZNF280A and SUHW2/ZNF280B) and a gene encoding a putative membrane glycoprotein (POM121L1).

Transcription
The NCBI database annotates five PRAME mRNA transcripts ranging from 2.1-2.7 kb in length (2141, 2162, 2197, 2220, 2776 bases) which encode the same protein.

Transcript (Including UTRs):
- Position: chr22:22890123-22896603, Size: 6481, Total exon count: 4
- Strand: -

Coding region:
- Position: chr22:22890489-22893484, Size: 2996, Coding exon count: 3

Pseudogene
The gene seems to have undergone multiple duplications during hominid evolution, and at least 22 PRAME-like genes and 10 pseudogenes have been identified in the human genome (Birtle et al., 2005).

Protein
Note
PRAME is a leucine-rich protein of which 21.8% of residues are leucine or isoleucine.
Predicted domain structure of the human PRAME sequence highlighting Leucine Rich Repeats (LRRs). The LRRs are numbered and indicated by the blue arrows; residues conserved in typical LRRs are highlighted in bold. The black boxes indicate regions predicted to have a high probability of $\alpha$-helicity, and two predicted NLS sequences are underlined. The boxed area in red is a region implicated in interaction with retinoic acid receptors (Wadelin et al., 2010).

**Description**
NP_006106 : Predicted 509 amino acid, 58 kDa protein.

**Expression**
PRAME is expressed at low levels in a few normal tissues, at intermediate level in adrenals, ovary, and endometrium and at high level in the testes. It has been shown to be overexpressed in malignant cells including the vast majority of primary and metastatic melanomas and is recognized by cytolytic T lymphocytes (Haqq et al., 2005). In PRAME-negative leukaemias the gene can be induced by demethylating agents (Sigalotti et al., 2004).

**Localisation**
The protein has been observed to localise to both the nucleus and perinuclear regions (Tajeddine et al., 2005). PRAME contains several candidate nuclear localisation signal (NLS) sequences (See Figure).

**Function**
Human PRAME and its paralogues are related to LRR family proteins, some of which are known to have functions in cell immunity and signal transduction. It has been suggested that, like TLRs, PRAME may be upregulated in response to encounters with microbial pathogens, and may be involved in targeting intracellular PAMPs to the Golgi for ubiquitylation and processing. (Wadelin et al., 2013). PRAME has been reported to function as a repressor of retinoic acid (RA) signalling through interactions with retinoic acid receptors (RARs) and repression of the RARb2 gene (Epping et al., 2005; Epping et al., 2007).

**Homology**
Sequence homology and structural predictions suggest that PRAME is related to the leucine-rich repeat (LRR) family of proteins such as the Toll-like receptors. The Oogenesins 1-4 also show considerable homology to PRAME and PRAME family members (Dade et al., 2003).

**Mutations**
Note
Some listed in COSMIC.

**Implicated in**

**Melanoma**
Note
High levels of PRAME mRNA are present in the majority of primary and metastatic melanomas (88% and 95% respectively) (Haqq et al., 2005), while being absent in normal haematopoietic tissues including bone marrow (Oehler et al., 2009; Radich et al., 2006; Steinbach et al., 2002; van Baren et al., 1998).

**Acute and chronic leukaemia, non Hodgkin's lymphomas**
Note
Numerous studies have reported highly elevated levels of PRAME in both acute and chronic leukaemias and non Hodgkin's lymphomas (van Baren et al., 1998; Matsushita et al., 2001; Oehler et al., 2009; Qin et al., 2009; Radich et al., 2006; Santamaria et al., 2008). PRAME expression has been suggested to be predictor for a good clinical outcome in childhood acute lymphoblastic
leukemia in addition to being a target for immunotherapy, and biomarker for the monitoring of minimal residual disease (Abdelmalak et al., 2014).

**Neuroblastoma**

**Note**

PRAME is expressed in high-stage neuroblastoma and associated with poor outcome (Oberthuer et al., 2004).

**Breast cancer**

**Note**

PRAME is expressed in advanced breast cancer and has been shown to be an independent prognostic factor for shortened disease-free survival (Doolan et al., 2008).

**Ovarian adenocarcinoma**

**Note**

PRAME was found to be a biomarker and prognostic factor for patients with stage III serous ovarian adenocarcinomas (Partheen et al., 2008).

**Lung squamous cell carcinoma**

**Note**

Northern blot analysis demonstrated that high proportion of positive tumours were shown to express PRAME (Ikeda et al., 1997).

**Head and neck cancer**

**Note**

Reverse-transcriptase polymerase chain reaction (RT-PCR) showed high expression of the gene coding for the tumor antigen PRAME in surgical samples of the tumors, margins, and lymph nodes from patients with a diagnosis of head and neck carcinoma (Figueiredo et al., 2006).

**Neurological neoplasms**

**Note**

PRAME has been identified as a potential CT antigen for medulloblastoma (Boon et al., 2003) and is universally expressed in high-stage neuroblastoma, and has been associated with poor outcome (Oberthuer et al., 2004).

**References**


Birtle Z, Goodstadt L, Ponting C. Duplication and positive selection among homin-specific PRAME genes. BMC Genomics. 2005 Sep 13;6:120


PRAME (preferentially expressed antigen in melanoma) Fulton J, Heery DM


This article should be referenced as such:

Leukaemia Section
Short Communication

dic(9;16)(p13;q11) PAX5/?

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Abstract
Short communication on dic(9;16)(p13;q11) PAX5/?, with data on clinics, and the genes implicated.

Clinics and pathology

Disease
Acute lymphoblastic leukemia (ALL)

Epidemiology
Only one case to date, a 15-year old boy with a pre-B-ALL (Coyaud et al., 2010).

Cytogenetics

Cytogenetics morphological
The dic(9;16) was the sole abnormality within a subclone.

Genes involved and proteins

PAX5
Location
9p13.2

Protein
391 amino acids; from N-term to C-term, PAX5 contains: a paired domain (aa: 16-142); an octapeptide (aa: 179-186); a partial homeodomain (aa: 228-254); a transactivation domain (aa: 304-359); and an inhibitory domain (aa: 359-391). Lineage-specific transcription factor; recognizes the consensus recognition sequence GNCCANTGAAGCGTGAC, where N is any nucleotide. Involved in B-cell differentiation. Entry of common lymphoid progenitors into the B cell lineage depends on E2A, EBF1, and PAX5; activates B-cell specific genes and repress genes involved in other lineage commitments. Activates the surface cell receptor CD19 and repress FLT3. Pax5 physically interacts with the RAG1/RAG2 complex, and removes the inhibitory signal of the lysine-9-methylated histone H3, and induces V-to-DJ rearrangements. Genes repressed by PAX5 expression in early B cells are restored in their function in mature B cells and plasma cells, and PAX5 repressed (Fuxa et al., 2004; Johnson et al., 2004; Zhang et al., 2006; Cobaleda et al., 2007; Medvedovic et al., 2011).

Result of the chromosomal anomaly

Hybrid gene
Description
Truncation of PAX5 after exon 5. The region in 16q11 does not contain any gene.

Fusion protein
Description
256 amino acids. The truncated protein contains the DNA binding paired domain and octapeptide of PAX5 (201 aa) and 55 aa from contiguous introns.
References


This article should be referenced as such:

**dic(9;17)(p13;q11) PAX5/TAOK1**

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**Abstract**

Short communication on dic(9;17)(p13;q11) PAX5/TAOK1, with data on clinics, and the genes implicated.

**Clinics and pathology**

**Disease**

Acute lymphoblastic leukemia (ALL)

**Epidemiology**

Only one case to date, a 3-year old girl with a pre-B-ALL (Coyaud et al., 2010).

**Cytogenetics**

**Additional anomalies**

There was a +X and additional rearrangements.

**Genes involved and proteins**

**PAX5**

**Location**

9p13.2

**Protein**

391 amino acids; from N-term to C-term, PAX5 contains: a paired domain (aa: 16-142); an octapeptide (aa: 179-186); a partial homeodomain (aa: 228-254); a transactivation domain (aa: 304-359); and an inhibitory domain (aa: 359-391). Lineage-specific transcription factor; recognizes the consensus recognition sequence GNCCANTGAAGCGTGAC, where N is any nucleotide. Involved in B-cell differentiation. Entry of common lymphoid progenitors into the B cell lineage depends on E2A, EBF1, and PAX5; activates B-cell specific genes and repress genes involved in other lineage commitments. Activates the surface cell receptor CD19 and repress FLT3. Pax5 physically interacts with the RAG1/RAG2 complex, and removes the inhibitory signal of the lysine-9-methylated histone H3, and induces V-to-DJ rearrangements. Genes repressed by PAX5 expression in early B cells are restored in their function in mature B cells and plasma cells, and PAX5 repressed (Fuxa et al., 2004; Johnson et al., 2004; Zhang et al., 2006; Cobaleda et al., 2007; Medvedovic et al., 2011).

**TAOK1**

**Location**

17q11.2

**Protein**

1001 amino acids (aa); from N-term to C-term, TAOK1 contains: a protein kinase domain (aa 28-281), a nucleotide binding site (aa 34-42), a poly-Glu stretch (aa 330-334), a Ser-rich region (aa 347-379), and coiled coil domains (aa 458-651; 754-877). Phosphorylation on Ser/Thr followed by Gln are the following: SQ motifs: aa 363-364; 554-555; 990-991, TQ motifs: aa 502-503; 643-644; 785-786 (Raman et al., 2007). TAOK1 is a serine/threonine-protein kinase. TAOK1 has the ability to phosphorylate MARK1 (MAP/Microtubule affinity-regulating kinase 1, 1q41), a kinase regulating microtubule dynamics and cell polarity (Timm et al., 2003). TAOK1 regulates apoptotic morphology via C-Jun N-terminal kinases (MAPK8, 10q11.22; MAPK9, 5q35.3; MAPK10, 4q21.3) and ROCK1 (Rho-associated, coiled-coil containing protein kinase 1, 18q11.1) (Zihni et al., 2006).
PAX5-TAOK1 translocation protein.

TAOK1 alters actin cytoskeletal organization and binds to microtubules, regulating their organization and stability (Zihni et al., 2006). The TAO kinases (TAOK1; TAOK2, 16p11.2; TAOK3, 12q24.23) mediate the activation of p38 (MAPK11, 22q13.33; MAPK12, 22q13.33; MAPK13, 6p21.31; MAPK14, 6p21.31) in response to various genotoxic stimuli. ATM (ataxia telangiectasia mutated, 11q22.3) phosphorylates the TAO kinases. TAO kinases are regulators of p38-mediated responses to DNA damage and are intermediates in the activation of p38 by ATM (Hutchison et al., 1998; Raman et al., 2007).

TESK1 (testis-specific kinase 1, 9p13.3) binds to and inhibits TAOK1. The elevation of TAOK1 results in microtubule disruption. SPRED1 (sprouty-related, EVH1 domain containing 1, 15q14) - TESK1 binding causes inhibition of TESK1, making F-actin fibers dynamic (Johne et al., 2008). Taok1 controls epithelial morphogenesis by promoting Fas2 (Fasciclin 2, the insect homologue of NCAM1 (neural cell adhesion molecule 1, 11q23.2)) endocytosis in Drosophila melanogaster (Gomez et al., 2012).

Result of the chromosomal anomaly

**Hybrid gene**

**Description**
Fusion of PAX5 exon 5 to TAOK1 intron 19-20, but in reverse orientation. Transcript with exon 1B to exon 5 of PAX5, and an additional sequence of 54 bp.

**Fusion protein**

**Description**
Predicted fusion protein of 218 amino acids. The predicted fusion protein contains the DNA binding paired domain of PAX5 (201 aa) and 17 amino acids.

**References**


Johne C, Matenia D, Li XY, Timm T, Balusamy K, Mandelkow EM. Spred1 and TESK1--two new interaction partners of the kinase MARKK/TAO1 that link the microtubule and actin cytoskeleton. Mol Biol Cell. 2008 Apr;19(4):1391-403


This article should be referenced as such:

Leukaemia Section
Short Communication

**t(3;12)(q26;p13) ETV6/MECOM / t(3;12)(q26;p13) ETV6/EVI1**

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Abstract

Short communication on t(3;12)(q26;p13) ETV6/MECOM, with data on clinics, and the genes implicated.

Identity

**Note**

Only a few cases were shown to involve a ETV6/MECOM fusion by FISH or RT-PCR. In other cases, only MECOM or ETV6 was shown to be rearranged by FISH. Over-expression of MECOM/EVI1 was found in other cases. Finally, no cytogenetic or molecular analysis was performed in a few cases.

Epidemiology

46 cases described so far; sex ratio: 29M/16F (1 unknown); age: 2.5-87 yrs (med: 49 yrs), unknown age for 10 cases.

Cytology

Dysplasia of megakaryocytes, multilineage involvement.

Prognosis

See survival curve above.

Cytogenetics

Cytogenetics molecular

Heterogeneity of the EVI1 breakpoints, as evidenced by the Cytocell Aquarius EVI1 Breakapart probe.

The EVI1 Breakapart probe contains three probes: a probe labeled in Aqua of 562 kb in size centromeric to the EVI1 gene, a probe labeled in Spectrum Green of 181 kb covering EVI1 and its flanking regions and a probe labeled in Spectrum Orange of 124 kb telomeric of the EVI1 gene (telomeric of MYNN and covering LRRC34).

Additional anomalies

Sole anomaly in 22 cases.

Additional anomalies: t(9;22)(q34;q11) in 5 cases, -7/del(7q) in 11 cases, del(5q) in 2 cases, others in 6 cases.
Survival curve (21 patients, Mean: 12 mths, Median: 10 mths).

Genes involved and proteins

MECOM
Location
3q26
Note
Alias EVI1
DNA/RNA
MECOM is a "complex entity" made of two genes, EVI1 and MDS1.
EVI1 has 16 exons, of which 14 are coding, the start ATG codons being in exon 3.
MDS1 has 4 exons, exon 4 being located in the vicinity of exon 1 of EVI1.
Splicing of the second exon of MDS1 to the second exon of EVI1 leads to a MDS1-EVI1 mRNA.
Protein
EVI1 contains two domains of seven and three zinc finger motifs separated by a repression domain and an acidic domain at its C-terminus.

ETV6
Location
12p13
Note
The ETV6 gene encodes a transcription factor frequently rearranged in myeloid and lymphoid leukemias.
DNA/RNA
The ETV6 gene spans a region of less than 250 kb at band 12p13.1 and consists of 8 exons. There are two start codons, one (exon 1a starting at codon 1) located at the beginning of the gene and another alternative (exon 1b starting at codon 43) upstream of exon 3.
Protein
The ETV6 protein (452 amino acids) contains two major domains, the HLH (helix-loop-helix) and ETS domains. The HLH domain, also referred to as the pointed or sterile alpha motif domain, is encoded by exons 3 and 4 and functions as a homodimerization domain. The ETS domain, encoded by exons 6 through 8, is responsible for sequence specific DNA-binding and protein-protein interaction.

Result of the chromosomal anomaly

Hybrid gene
Description
Two mechanisms for generating the fusion gene, depending upon the involvement of the MDS1 gene.
Transcript
1) In-frame transcript consisting of the first two exons of ETV6 fused to MDS1 sequences, which in turn is fused to the second exon of the EVI1 gene.
2) Out-of-frame fusion by direct fusion between exon 2 of ETV6 and exon 2 of EVI1, but keeping the open reading frame of EVI1 intact.

**Fusion protein**

**Description**
ETV6 contributes no known functional domain to the predicted chimeric protein but functional domains of EVI1 are retained.

**Oncogenesis**
The oncogenic potential of the translocation could be the result of the ETV6 promoter driving the transcription of EVI1. Because the ETV6 promoter is active in hematopoietic cells, this would result in inappropriate expression of the transcription factor EVI1.

**References**


Nucifora G. The EVI1 gene in myeloid leukemia. Leukemia. 1997 Dec;11(12):2022-31


This article should be referenced as such:
t(6;20)(q13;q12) LMBRD1/CHD6

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Abstract
Short communication on t(6;20)(q13;q12) LMBRD1/CHD6, with data on clinics, and the genes implicated.

Clinics and pathology

Disease
Myelodysplastic syndrome/myeloproliferative disorder (MDS/MPD) in transformation (Acute myelocytic leukemia - AML)

Epidemiology
No cases registered in the Mitelman database.

Clinics
A 78-year-old woman seen because of worsening of cytopenias two years following diagnosis of MDS/MPD. No further investigations.

Evolution
Patient deceased soon after diagnosis of AML.

Cytogenetics

Note
The t(6;20)(q13;q12) involves two genes, the LMBRD1 and CHD6 genes, that have never been shown to form a fusion gene.

Cytogenetics morphological

The t(6;20)(q13;q12) is identified by banding cytogenetics.

RHG banding showing chromosomes 6 and 20 and the derivatives der(6) and der(20).

Cytogenetics molecular

To determine the position of the breakpoints on chromosomes 6 and 20, BACs located in the bands of interest were used as probes in FISH experiments. Analysis with RP11-359N1 showed that one signal hybridized to the normal chromosome 6, and the other split and hybridized to both der(6) and der(20). Analysis with RP11-257H6 showed that one signal hybridized to the normal chromosome 20, and the other split and hybridized to both der(6) and der(20). Co-hybridization with both BAC clones showed two fusion signals. RP11-359N1 contains the LMBRD1 gene and RP11-257H6 the CHD6 gene.
FISH with BACs RP11-359N1 (spectrum green, located in 6q13 and containing LMBRD1) and RP11-257H6 (spectrum orange, located in 20q12 and containing CHD6) showing co-hybridization.

Genes involved and proteins

**LMBRD1**

Location  
6q13  

Note  
Mutations in the LMBRD1 gene is responsible for methylmalonic aciduria and homocystinuria type cbLF (autosomal recessive disorder). It is a disorder of cobalamin metabolism characterized by decreased levels of the coenzymes adenosylcobalamin and methylcobalamin due to accumulation of free cobalamin in lysosomes, thus preventing its conversion to cofactors. Clinical features include poor feeding, failure to thrive, developmental delay, stomatitis, glossitis, seizures, macrocytic anemia, neutropenia, thrombocytopenia and methylmalonic aciduria responsive to vitamin B12.

DNA/RNA  
The LMBRD1 gene contains 15 exons, spanning 115 kb. Three transcripts (splice variants) are known, two being protein coding.

Protein  
The protein has 540 amino acids (61.4 kDa) and localizes to the lysosome membrane. It contains nine transmembrane helices and six putative glycosylation sites with an N terminus in the lysosomal interior and a cytoplasmatic C terminus. It is a probable lysosomal cobalamin transporter, being required to export cobalamin from lysosomes, which in turn allows its conversion to cofactors. It also appears to play a key role in mediating and regulating the endocytosis of the insulin receptor. Isoform 3 coding the nuclear export signal interacting protein (NESI) may play a role in the assembly of hepatitis delta virus.

**CHD6**

Location  
20q12  

Note  
The CHD6 gene, located 403 kb centromeric to STS marker D20S108 is constantly deleted in MDS and MPD associated with del(20q) or ider(20q).

DNA/RNA  
The CHD6 gene contains 37 exons of which 36 are coding, spanning 216 kb. Nine transcripts are known.

Protein  
The gene encodes a member of the SNF2/RAD54 helicase protein family. The protein has 2715 amino acids (305 kDa) and localizes to the nucleus. It contains two chromodomains, a helicase domain, and an ATPase domain. The protein is thought to be a core member of one or more of chromatin remodelling complexes. It may function as a transcriptional repressor. It is involved in the cellular repression of influenza virus replication and in transcriptional repression of papillomavirus.
References


Marfella CG, Imbalzano AN. The Chd family of chromatin remodelers. Mutat Res. 2007 May 1;618(1-2):39-40


This article should be referenced as such:

Predicted human structural clusters of miRNAs target cancer genes

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Abstract
Deregulation of gene expression is one of the main characteristics of cancer cells. The implication of microRNAs (miRNAs), a class of small non-coding RNAs implicated in post-transcriptional regulation of gene expression, in this process have rapidly become evident. As protein-coding genes, miRNAs can act as tumor suppressors or oncogenes (we speak about oncomirs). Recent studies have highlighted the paralogous clusters of miRNAs mir-17-92 and mir-106a-363 to be involved in carcinogenesis. It features the importance of a local and structural organization of miRNAs with potential impact on cancers. We performed computational predictions of structural clusters of miRNAs sharing the same characteristics as the two previously described clusters at the human genome scale. We show a functional organization of miRNAs in structural clusters where the predicted miRNA targets are enriched for cancer associated genes. On the other hand, we also show co-localization of structural clusters of miRNAs with genes involved in signaling pathways known to be disrupted in cancer. Taken together, the results provide new insights into the organization of miRNAs in the human genome along with their potential impact on carcinogenesis.

Introduction
MicroRNAs (miRNAs) are a class of endogenous ~18-25nt-long RNAs involved in post-transcriptional regulation in animals and plants. They play crucial functions in cell physiology and ensure plethora of key cellular processes by negatively regulating expression of target genes. While computational software usually predicts miRNAs on a one to one basis, recent studies have shown that they can be organized into clusters, sharing similar biological functions. Indeed, miRNAs can group together along the human genome to form stable secondary structures made of several hairpins hosting miRNAs in their stems. Alignment of miRNA sequences lying within the same cluster or in different clusters revealed a significant number of miRNA paralogs shared among and within clusters, implying an evolution process targeting the potentially conserved roles of these molecules. In this report, we define structural clusters of miRNAs as genomic regions typically smaller than 1-2 kb and which folds into a secondary structure presenting several hairpins, where miRNAs are located on their stems. The known miRNA clusters mir-17-92 (Hayashita et al., 2005) and mir-106a-363 (Landais et al., 2007) satisfy such structural conditions, and we looked for others having the same characteristics in the human genome. Notice that miRNA clusters mir-17-92 and mir-106a-363 are known to play a role in human tumour development.
We report here the results described in Mathelier and Carbone, 2013 by focusing on the potential involvement of structural clusters of miRNAs in cancer. Using newly developed computational methods, we predicted structural clusters of miRNAs at the human genome scale. The predictions were made using three different approaches: (1) finding structural clusters of paralogous miRNAs from the genomic sequence only, (2) constructing structural clusters of miRNAs predicted using small RNA-seq (sRNA-seq) data, (3) combining paralogous miRNAs prediction and sRNA-seq data. The precursors of miRNAs (pre-miRNAs) have been predicted using the MIReNA tool (Mathelier and Carbone, 2010), which has been described as a first-choice when predicting new miRNAs in mammals (Li et al., 2012). Predictions were validated a posteriori as bona fide miRNAs by analysing expected characteristics of miRNAs and pre-miRNAs. We highlighted that structural clusters of miRNAs co-localized with genes related to signal transduction pathways, known to be involved in carcinogenesis. Finally, a functional analysis of potential targets of the predicted miRNAs confirms a regulatory role of most predicted miRNAs in structural clusters and highlights their potential involvement in cancer.

I. Overview of predicted miRNA structural clusters

The computational tool developed in Mathelier and Carbone, 2013 predicts structural clusters either by looking for repeated sequences in palindromic regions, by using deep-sequencing reads as potential miRNAs forming structural clusters or by combining the two kinds of information (see Methods section of Mathelier and Carbone, 2013 for details). While the first strategy is based on the human genomic sequence to make predictions, the two other strategies are using data coming from the small RNA-sequencing technology. We want to stress here that the sRNA-seq data sets used are mainly derived from cancer cells. Namely, breast cancer cells, melanoma and pigment cells, and cervical cancer cells were used for predicting structural clusters of miRNAs. As the computational method development is based on the characteristics of the two already known miRNA structural clusters, the newly predicted structural clusters display similar characteristics to the known mir-17-92 and mir-106a-363 clusters.

When applied to the human genome, we predicted 416 structural clusters containing 1713 miRNAs (see Figure 1 for examples). To validate a posteriori the predicted miRNAs as bona fide miRNAs, we highlighted that 70% of miRNA predictions based on either sequence analysis or deep-sequencing data contain seeds (i.e. subsequences corresponding to positions 2-8 in the miRNA) identical to known miRNAs (see Table 1). The presence of already identified seeds in miRNAs of structural clusters increases the level of confidence in the predictive approach as they represent a signature of target prediction (Lewis et al., 2003; Lewis et al., 2007). Furthermore, a very large fraction of structural cluster sequences predicted from deep-sequencing data, and 10% of those predicted using paralogs contain at least one known miRNA sequence (100% identity is asked) from miRBase; many of these miRNAs being human miRNAs.
When considering predictions obtained from sRNA-seq data, we discovered 12 structural clusters containing miRNAs that are all mapped by reads coming from the same deep-sequencing experiment: eight structural clusters belong to a data set from cervical cancer cells and the others to melanoma and pigment cells. Predictions were made by combining all sRNA-seq data sets together. Note that the known mir-17-92 and mir-106a-363 structural clusters could only be predicted by combining sRNA-seq reads coming from multiple experiments. Indeed, miRNAs hosted in the stems of the structural clusters appeared to overlap with reads from specific experiments. Namely, four predicted miRNAs over five in mir-17-92 and four over six in mir-106a-363 come from the same experiment. It highlights that even though miRNAs are organized in clusters, their expression can be cell-type/tissue specific. This evidence supports search criteria that mix together reads coming from different experiments.

Our predictions highlight that most of the 99 predicted structural clusters are only partially processed under specific conditions. Indeed, 75 of the 99 predicted structural clusters obtained from sRNA-seq contain at least two miRNAs coming from the same experimental data set, 22 contain at least three, six contain at least four and one contain at least five.

II. miRNA structural clusters co-localize with genes associated to specific signal transduction pathways involved in carcinogenesis

We studied whether structural clusters of miRNAs were functionally organized along the human chromosomes. We hypothesized that genes sharing similar biological functions with miRNA clusters might co-localize along the human genome, allowing for an improved transcriptional efficiency. To evaluate this hypothesis, we analyzed larger and larger regions (of a few million bases) around the predicted structural clusters and counted the amount of genes, in the regions, involved in specific biological pathways. All subclasses and pathways from all KEGG's pathways have been analyzed. We showed that groups of genes involved in specific biological pathways and structural clusters of miRNAs co-localize in a statistically significant manner along the human chromosomes. For instance, immune system diseases and sensory system subclasses are highlighted.

We further explored genes involved in signal transduction pathways as they have been recurrently shown to be involved in cancer development and have been defined as preferential targets for cancer therapy (Bode and Dong, 2005; Reddy and Couvreur, 2010). While taken all together, the signal transduction pathways are not significantly co-localizing with structural clusters. Nevertheless, some specific pathways are enriched in structural cluster regions. Namely, the Wnt (150 genes, P =0.015), Notch (47 genes, P =0.017), and Hedgehog (56 genes, P =0.048) pathways display a non-random gene distribution in structural cluster regions (see Figure 2). These signal transduction pathways were previously pinpointed as prime candidates for miRNA-mediated regulation, and several examples were reported to suggest miRNAs to be generators of graded responses or amplifiers in signal pathways, both for single pathways or signalling cross-talks (Inui et al., 2010). Notice that previous works highlighted the role of the Wnt (see Polakis, 2000; Anastas and Moon, 2013 for reviews), Notch (Hu et al., 2012; Al-Hussaini et al., 2012; Lobry et al., 2011), and Hedgehog (Amakye et al., 2013; Gupta et al., 2010) pathways in cancer development and all three pathways have been described as preferred therapeutic targets for cancer treatment.
Figure 2: Curves relating coverage of structural cluster regions to genes belonging to specific KEGG’s pathways: Wnt, Notch and Hedgehog. The curve generated by randomly selecting genes is also plotted. Similar curves are reported in the Supplementary Material of Mathelier and Carbone, 2013 for all statistically significant analysis of KEGG’s subclasses.
Figure 3: KEGG pathways containing genes whose 3'UTRs are targeted by some predicted miRNAs. Functional analysis is realized on a set of miRNA/3'UTR pairs (see Mathelier and Carbone, 2013). A. KEGG pathways showing a Benjamini corrected p-value < $5 \times 10^{-4}$ are drawn. B. KEGG pathways showing a Benjamini corrected p-value < 0.05 and associated to genes co-localizing with structural clusters (p-value < 0.05, see previous section) are drawn. A-B. Each node of the graph represents an enriched KEGG pathway where the size of the node is proportional to the number of targeted genes (from 45 to 237 genes in A and from 37 to 188 genes in B). The higher the opacity of the nodes, the lower the associated p-value. An edge between two nodes indicates that the pathways are sharing genes. The larger the width of the edge, the larger the number of shared genes (from 3 to 127 in A and from 4 to 55 in B). Cancer KEGG pathways are painted in red, signaling pathways are painted in green, and other pathways are painted in grey. See Mathelier and Carbone, 2013 for a full table reporting also GO terms and PIR keywords Swiss-Prot Database analyses.
III. miRNAs in structural clusters target cancer genes

We further our functional analysis of miRNAs contained in computed structural clusters by predicting their potential targets. Using the miRanda (John et al., 2004) and PITA (Kertesz et al., 2007) tools, we predicted targets for all the miRNAs computed in the structural clusters in 3'UTR and CDS of human genes (see Methods of Mathelier and Carbone, 2013 for details). The following results have been obtained by using predictions from miRanda but very similar results are derived from PITA’s predictions.

A Gene Ontology (GO) functional enrichment analysis of the genes targeted shows that almost half (43%) of the biological processes (BP) GO terms associated to miRNA/3'UTR pairs are involved in regulation and have the motif 'regulation of' in their name (see Mathelier and Carbone, 2013). It suggests that predicted miRNAs might be involved in the degradation of transcription factors, as it is the case for the two already known miRNAs of chromosome 13 (mir-17-92 structural cluster) regulating protein E2F1 and being regulated by c-Myc that also regulates E2F1 (O'Donnell et al., 2005).

When considering KEGG pathways, one of the pathways with the most enriched gene set in miRNA targets is 'pathways in cancer' (P< 5.4e-9). Moreover, we observe 14 pathways corresponding to different types of cancer ranked as statistically significant among all KEGG pathways (see Figure 3A and Oxford Journals). Notice that the 'melanogenesis' (P< 1.1e-5) and the 'melanoma' (P< 2.8e-5) pathways are highlighted. These specific result needs to be considered in the context of the set of sRNA-seq data sets that we used with several skin cell derived data. Moreover, 115 miRNAs (20%) targeting 3'UTRs associated to melanogenesis and melanoma over a total of 550 are contained in structural clusters identified using deep sequencing data derived from corresponding data sets. The enrichment for targets involved in melanogenesis and melanoma associated to miRNAs predicted from skin sRNA-seq data reinforces our confidence in predicting structural clusters of bona fide miRNAs using deep sequencing reads. Finally, we observed significant enrichment for pathways already known to be involved in carcinogenesis like apoptosis or important signalling pathways such as P53, Wnt, MAPK, Hedgehog, mTOR, VEGF, and Notch (see Oxford Journals). Notice the enrichment of signaling pathways which have been previously highlighted as co-localizing with miRNA structural clusters (see Figure 3B), the subsequent results show that they are also potential targets of these miRNAs.

Using a more stringent set of predicted targets (by lowering the threshold on miRNA/target predictions, (see Mathelier and Carbone, 2013 for details), we observe a stronger signal with BP GO terms associated to 'melanogenesis' and 'melanoma' over a total of 550 contained in structural clusters identified using deep sequencing data derived from corresponding data sets. The enrichment for targets involved in melanogenesis and melanoma associated to miRNAs predicted from skin sRNA-seq data reinforces our confidence in predicting structural clusters of bona fide miRNAs using deep sequencing reads. Finally, we observed significant enrichment for pathways already known to be involved in carcinogenesis like apoptosis or important signalling pathways such as P53, Wnt, MAPK, Hedgehog, mTOR, VEGF, and Notch (see Oxford Journals). Notice the enrichment of signaling pathways which have been previously highlighted as co-localizing with miRNA structural clusters (see Figure 3B), the subsequent results show that they are also potential targets of these miRNAs.

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signalling pathways are among the identified ones, as already pointed out in the functional analysis of structural clusters regions.

It has been previously shown that miRNAs can also target genes by binding to CDS regions and not only to 3'UTRs (Tay et al., 2008). Looking for target predictions within CDSs, the analysis confirmed the observations already pointed out for 3'UTR targets and highlighted the same statistically significant terms on different data sets. From KEGG's pathways, we observe 'pathways in cancer' as the first highlighted term followed by specific cancers, signalling pathways and several cardiomyopathies (see Oxford Journals).

It is important to highlight that the miRNA/targets predictions ask for a high miRNA/target binding energy and that we observe a tendency for miRNAs targeting genes from the same functional class to be localized in the same structural clusters (see Table 2). For instance, when considering structural clusters with at least one miRNA targeting genes in KEGG 'pathways in cancer' we observe that for ~50% of these clusters, all the miRNAs are targeting genes in these pathways. Furthermore, the functional analysis on miRNAs predicted by paralogous sequences and by deep-sequencing data taken separately, provides comparable results to those described earlier in the text. It shows that miRNAs predicted from a specific methodology are not biasing the functional target analysis. Finally, among targets obtained from deep-sequencing data, we observe a stronger signal of pathways in 'cancer' and 'melanogenesis' obtained for the KEGGs data set in agreement with the usage of reads coming from melanoma cancer cells.

Conclusion

The discovery of structural clusters mir-17-92 and mir-106a-363 involved in cancer development provided the need for a computational tool that helps to characterize potential structural clusters within the human chromosomes as new candidates for experimental tests. In Mathelier and Carbone, 2013, we predicted structural cluster of miRNAs. Predictions were made following three different methodologies: (1) using paralogous miRNAs derived from genomic sequence analysis, (2) predicting miRNAs/pre-miRNAs using sRNA-seq data, and (3) combining the two previous methods. Predictions of structural clusters based on deep sequencing data are showing that 86% of them contain miRNAs with known seeds. We highlighted 13 new structural clusters whose miRNAs are identified by reads occurring either in cervical cancer or in melanoma and pigment cells experiments.

We showed that structural cluster regions (i.e. genomic regions surrounding structural clusters) are enriched for genes involved in cancer-related pathways such as the Wnt, Hedgehog, and Notch pathways.

A target genes functional analysis strongly supports a regulatory role of most predicted miRNAs and, notably, a strong involvement of predicted miRNAs in the regulation of cancer pathways.

Our findings highlight miRNA regulation mechanisms (potentially affected by mutation) as potential causes of signalling pathway disfunctioning. Experiments for testing the in silico gene target identification are required.

Appendix

The predictions of structural clusters miRNAs were originally made using the hg18 version of the human genome.

The localization of the predicted miRNAs/pre-miRNAs have been lifted over to the hg38 version of the human genome and can be found as .bed files at UPMC.

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