Leukaemia Section

Review

del(X)(p22p22) P2RY8/CRLF2
del(Y)(p11p11) P2RY8/CRLF2

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Published in Atlas Database: March 2017
Online updated version : http://AtlasGeneticsOncology.org/Anomalies/delXp22PR2Y8-CRLF2ID1599.html
Printable original version : http://documents.irevues.inist.fr/bitstream/handle/2042/68761/03-2017-delXp22PR2Y8-CRLF2ID1599.pdf
DOI: 10.4267/2042/68761

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Abstract

Review on del(X)(p22.33p22.33) or del(Y)(p11.32p11.32), with data on the genes involved, and clinics.

KEYWORDS
PR2Y8/CRLF2, PAR1 deletion, Ph-like, BCR-ABL1-like ALL, B cell precursor Acute Lymphoblastic Leukemia

Identity

Other names
PR2Y8/CRLF2 rearrangement, PR2Y8/CRLF2 fusion, PAR1 deletion

Clinics and pathology

Disease
Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like (Arber et al., 2016)

Etiology
Among positive patients the PR2Y8/CRLF2 rearrangement has been detected in different proportions of cells, in principal clones and in minor subclones. Original major clones may persist, disappear, or revert to a minor clone; the minor clones may evolve into a major clone or loss, continue or substitute by a clone with different breakpoint. Based on this, it has been proposed that PR2Y8/CRLF2 is not a driver abnormality in the process of leukemogenesis. Instead, it is a secondary lesion and requires concomitant mutations to become functionally important. The presence of acquire CRLF2 rearrangements in constitutional trisomy 21, and the coexistence of the PR2Y8/CRLF2 and iAMP21 in non-Down syndrome B-cell precursor (BCP) acute lymphoblastic leukemia (ALL), also suggest that CRLF2 rearrangements requires cooperating mutations to activate JAK/STAT and PI3K/mTOR pathways (Morak et al., 2012).

Epidemiology
PR2Y8/CRLF2 positive patients are included in the Ph-like BCP-ALL subgroup, based on its characteristic gene expression signature (Arber et al., 2016). The prevalence of this subgroup is associated with age, high-risk stratification and ethnicity (Harvey et al., 2010). It presents in about 10% of
standard-risk childhood ALL, and in >25% of young adults; CRLF2 is the most frequent deregulated gene in the Ph-like subgroup. Overexpression of CRLF2 results in constitutive JAK/STAT activation and cytokine-independent growth (Roberts et al., 2015). In the total of BCP-ALL patients 6% present overexpression of CRLF2, this deregulation is more prevalent among Down syndrome patients (54%). In an unscreened cohort of pediatric patients, the Medical Research Council (MRC) found predominance of PR2Y8/CRLF2 (5%) in contrast to IGH/CRLF2 (1%). In comparison, the Children's Oncology Group (COG) observed that IGH/CRLF2 is twice as prevalent as PR2Y8/CRLF2 (Ensor et al., 2011); this variability could be explained by the selection of high risk ALL patients analysed in this study, including a high proportion of older patients and Hispanic patients, who are 5 times more possible to have CRLF2 abnormalities compared with patients with other ethnicities. In children with BCP-ALL and Down syndrome PR2Y8/CRLF2 was identified in 54.5% of cases (Mullighan et al., 2009). In adults with BCP-ALL the prevalence of Ph-like is >20%, of this group, the IGH/CRLF2 (57.6%) is more prevalent than PR2Y8/CRLF2 (21.2%) (Roberts et al., 2017). In general, IGH/CRLF2 patients are older than PR2Y8/CRLF2 patients.

**Clinics**

ALL with BCP immunophenotype with rearrangement involving a cytokine receptor, included in the BCR-ABL1-like ALL subgroup.

**Treatment**

The PR2Y8/CRLF2 rearrangement results in kinase-activating lesion that causes the constitutive activation of the JAK/STAT signalling pathway. Therefore, patients with PR2Y8/CRLF2 ALL could benefit from the treatment with clinically approved tyrosine kinase inhibitors such as Ruxolitinib, which target these pathways. The treatment of these patients with the relevant tyrosine kinase inhibitor could potentially improve their survival (Harrison, 2013).

**Prognosis**

In different cohorts of children with high risk ALL, the presence of CRLF2 alterations have been associated with poor clinical outcome. However, not in all the study groups have been found positive correlations. The different treatment protocols, not standardized methods for diagnosis of CRLF2 alterations or over-expression, and limited prospective studies for analysing prognostic value are source of bias. A meta-analysis study including 5945 patients of seven different studies analysed the association between CRLF2 alterations and survival of children with ALL, the results showed that CRLF2 deregulation predicts poor prognosis. In adults, the outcome of patients with Ph-like ALL is inferior compared with the outcome of patients with non-Ph-like ALL, excluding BCR/ABL1 and MLL positive cases (Roberts et al., 2017).

It is expected that PR2Y8/CRLF2 positive clones contribute to disease progression. However, in children it has been found heterogeneity in PR2Y8/CRLF2 clone sizes, and no participation in relapses if the size is very small at diagnosis. In part, these may be the basis of the controversies about the prognostic value of this rearrangement and the over-expression of CRLF2 as risk markers. A revision of these parameters should be done.

**Disease**

T-lymphoblastic leukemia/lymphoma (T-ALL)

**Note**

It has been reported CRLF2 over-expression in T-ALL patients; however, PR2Y8/CRLF2 has not been found in these cases. Deregulation of CRLF2 in this entity is considered a poor prognosis marker (Palmi et al., 2016).

**Cytogenetics**

**Cytogenetics molecular**

del(X)(p22p22); del(Y) P2RY8/CRLF2

PR2Y8/CRLF2 is a cryptic rearrangement, results from intrachromosomal deletions within the pseudoautosomal region (PAR1) located in the p arm of the sex chromosomes (Harrison, 2013). Until now, this abnormality has not been found with the recurrent cytogenetic abnormalities commonly observed in BCP-ALL, but can occur with the iAMP21 or in high hyperdiploid patients; one patient has been reported with the coexistence of PR2Y8/CRLF2 and IGH/CRLF2 (Harvey et al., 2010). A novel CRLF2 rearrangement formed with a gene of the PAR1 region has been described (CSF2RA/CRLF2) (Yano et al., 2015). The IKZF1 deletions may be also observed, but are less prevalent than IGH/CRLF2 (Ensor et al., 2011). JAK1, JAK2 and JAK3 mutations are found in 10 % of high-risk BCP-ALL cases (Mullighan et al., 2009).

In Down syndrome patients, +X, and del(9)(p22) have been found in PR2Y8/CRLF2 positive cases (Figure 1). JAK mutations are present up to 28% of patients; JAK2 mutations in the pseudokinase domain are the most common (Mullighan et al., 2009).

FISH in interphases with the PR2Y8/CRLF2 (PAR 1) deletion probe (Cytocell Aquarius) on a bone
marrow sample of a female patient with Down syndrome. A) The expected two normal signals (red/green): B) One normal signal (red/green) and one corresponding to the PAR1 deletion (green with white arrow). C) One normal signal (red/green) and two abnormal signals (green with white arrows) which indicate the presence of two X chromosomes with the PAR1 deletion.

**Genes involved and proteins**

**CRLF2 (cytokine receptor-like factor 2)**

**Location**

Xp22.33

**Note**

It is located at the PAR1 of chromosome X, Xp22.33, and chromosome Y, Yp11.

**Other names and symbols:** Thymic Stromal Lymphopoietin Protein Receptor, TSLP Receptor, IL-XR, TSLPR, CRL2, Thymic Stromal-Derived Lymphopoietin Receptor, Cytokine Receptor-Like 2.

**DNA/RNA**

The genomic location of CRLF2 gene in chromosome X starts in 1,187,549 bp from pter, and ends in 1,212,815 bp from pter. The size is 25,267 bases, with minus strand orientation NC_018934.

**Protein**

The cytokine receptor-like factor 2 has a size of 371 amino acids, with a molecular mass of 42,013 Daltons. Cytokine receptor-like factor 2 is a transmembrane protein with an extracellular domain of 210 residues, and an intracellular domain of 119 residues (Zhang et al., 2001). CRLF2 heterodimerizes with IL7R constituting a functional receptor (Rochman et al., 2010; Bugarin C et al., 2015).

**Somatic mutations**

The CRLF2 711T>G (Phe232Cys) mutation has been found in ALL blasts. The CRLF2 Phe232 residue is near to the junction of the extracellular and transmembrane domains. The Phe232Cys mutation confers a constitutive dimerization through the cysteine residues inducing the growth of cells. This mutation also promotes the up-regulation of transcriptional targets downstream of the JAK/STAT pathway (Yoda et al., 2010).

**P2RY8 (purinergic receptor P2Y, G-protein coupled, 8)**

**Location**

Xp22.33

**Note**

P2RY8 is located at the pseudoautosomal region 1 (PAR1) of chromosome X, Xp22.33 and chromosome Y, Yp11.

**Other names and symbols:** purinergic receptor P2Y, G-protein coupled, 8, P2Y8, G-protein coupled purinergic receptor P2Y8, P2Y purinoceptor 8.

**DNA/RNA**

The genomic location of P2RY8 gene in chromosome X starts in 1,462,572 bp from pter and ends in 1,537,506 bp from pter. The size is 74,935 bases, with minus strand orientation NC_000023.
Protein
The P2Y purinoceptor 8 protein has a size of 359 amino acids, with a molecular mass of 40635 Daltons.

Result of the chromosomal anomaly

Hybrid gene

Note

The PR2Y8/CRLF2 rearrangement results from an interstitial microdeletion involving the PAR1, which is located in the short arm of both sex chromosomes. This deletion causes the CRLF2 overexpression through the juxtaposition of the entire coding sequence of CRLF2 with the first non-coding exon of P2RY8, and their transcriptional control elements that are highly active in lymphoid cells (Morak et al., 2016; Vesely et al., 2016; Ensor et al.; 2016; Mullighan et al; 2009).

Description
The PAR1 deletion measures approximately 320 Kb and deletes the complete coding sequence of P2RY8 along with ASMTL, SLC25A6, IL3RA and CSF2RA genes, bringing together the first exon of P2RY8 to exon 1 of CRLF2. The breakpoints of the deletion are highly conserved and are located 3.4 Kb upstream of the CRLF2 exon 1 and 0.3-1 Kb distal to P2RY8 exon 1 (Mullighan et al., 2009). Two new alternative breakpoints have been described, 2.1 Kb and 0.1 Kb upstream of CRLF2 exon 1, but in all cases the deletion place the full CRLF2 open reading frame under transcriptional control of the P2RY8 promoter (Morak et al., 2016). The presence of recombination signal sequences immediately internal to the deletion breakpoints suggests that the PAR1 deletion may arise as a result of aberrant activity of the RAG recombinases, this phenomenon has been described for other chromosomal rearrangements in B- ALL (Mullighan et al; 2009).

Transcript
The chimeric transcript contains the 5-UTR region of P2RY8 joined to exon 1 of CRLF2, prior to the transcription start codon (Hertzberg et al., 2016). The PR2Y8 5-UTR region measures approximately 226 bp, and the open reading frame of CRLF2, which is approximately of 1116 bp, remains complete; therefore the chimeric transcript size is 1342 bp (Yap et al., 2016).

Detection
The PR2Y8/CRLF2 positive cases can be detected at genomic level by long range PCR or MLPA techniques using genomic DNA extracted from leukemic cells, however, these assays only reveal the presence of the rearrangement. On the other hand, FISH assay informs about the proportion of abnormal cells, it has been designed deletion probes for covering the telomeric end of P2RY8 and a region distal to the gene, and a control probe that covers a region of the proximal side of P2RY8 (Morak et al., 2016). At RNA level, RT-PCR can confirm the presence of chimeric transcripts. The overexpression of CRLF2 can be measured by qRT-PCR using different methods of absolute and relative measurement (Mullighan et al; 2009).

Fusion protein

Oncogenesis
The overexpression of CRLF2 produced by the PR2Y8/CRLF2 rearrangement causes the constitutive activation of JAK/SAT signaling and the PI3K/mTOR, Ras, MAPK and Bcl-2 downstream related pathways in the leukemic blast. These signaling pathways have a central role in the regulation of the cell proliferation, survival, differentiation and immune response; hence its malfunction contributes to the leukemic transformation (Roberts KG et al., 2015).

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This article should be referenced as such: Juanve-Valézquez R, Martínez-Anaya D, Pérez Vera P, del(X)(p22p22); del(Y) P2RY8/CRLF2. P2RY8/CRLF2. Atlas Genet Cytogenet Oncol Haematol. 2018; 22(2): 52-56.