RRM1 (ribonucleotide reductase M1)
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Identity
Other names: R1; RIR1; RR1
HGNC (Hugo): RRM1
Location: 11p15.4
Local order: STIM1 - RRM1 - OR55B1P - LOC643244.
Note: The RRM1 gene is oriented on the plus strand. The chromosomal region containing the RRM1 gene locus displays frequent loss of heterozygosity (LOH) in lung cancer and is amplified in some instances of acquired resistance to gemcitabine and hydroxyurea.

DNA/RNA

Description
The RRM1 gene consists of 44182 base pairs which encode 19 exons.

Transcription
The mature RRM1 mRNA is 3234 ribonucleotides in length and harbors an open reading frame of 2379 ribonucleotides.

Pseudogene
There are no reported human RRM1 pseudogenes.

Protein

Note
The RRM1 protein functions in a heterodimeric tetramer with either RRM2 (RRM2a) or p53R2 (RRM2b) in the ribonucleotide reductase holoenzyme. While this interaction is essential for the de novo synthesis of deoxyribonucleotides, it is unclear whether this association is necessary for other biological activities ascribed to RRM1.

Description
The RRM1 protein is 792 amino acids in length and has a calculated molecular weight of 90 kDa. Analysis of the human RRM1 protein via the NCBI Conserved Domains program reveals an N-terminal ATP-cone domain and a Ribonucleotide Reductase (RNR) Class I domain which spans the majority of the protein. The RNR Class I domain harbors the all-alpha domain, the barrel domain, as well as regions and residues comprising the active site, the allosteric effector dTTP-binding site, the RRM2 peptide-binding site and the dimer interface.

Expression
While the RRM2 subunit of the ribonucleotide reductase holoenzyme is regulated in a cell cycle-specific manner, RRM1 expression remains relatively constant in actively proliferating cells. However, expression of RRM1 is significantly decreased upon exit from the cell cycle to G0 or terminal differentiation. This decreased level of RRM1 expression in non-dividing cells is essential for the supply of dNTPs for mitochondrial DNA replication. Upregulation of RRM1 occurs when cells are stimulated to re-enter the cell cycle from G0.

Localisation
Depending on the experimental technique employed, the cell line or tissue sample, the cellular state and the antibody source, RRM1 can be detected within the nucleus, the cytoplasm or both. In quantitative immunofluorescent staining of non-small-cell lung cancer (NSCLC) cell lines and tumor samples, RRM1 protein is predominantly nuclear. The method of analyzing nuclear RRM1 expression in resected NSCLC tumor samples can be used for survival prognostication. This is in agreement with fractionation of NSCLC cell lines, where RRM1 is detected almost
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exclusively in the nuclear fraction. In contrast, studies utilizing various experimental methods to examine RRM1 in mouse and human lung fibroblasts find RRM1 to be cytoplasmic. This is in line with other studies that find RRM1 localized in cytoplasmic compartments in mouse fibroblasts, as well as cytoplasmic RRM1 immunoreactivity in various rat tissues. Still, another study demonstrates both nuclear and cytoplasmic RRM1 in multiple cell lines and finds RRM1 localized to the nuclear membrane, while others report a DNA damage-dependent relocalization of RRM1 from the cytoplasm to the nucleus. Given the current evidence, it is likely that RRM1 localization is dependent on cell type, tissue of origin, and cellular state. It is also evident that variables such as the antibody and experimental technique employed obscure a conclusive answer as to the true localization characteristics of RRM1.

Function

The RRM1 protein is implicated in deoxyribonucleotide (dNTP) synthesis, DNA damage response and repair and tumor suppression. In dNTP synthesis, RRM1 serves as the regulatory subunit of the ribonucleotide reductase (RR) holoenzyme, which catalyzes the de novo synthesis of dNTPs from ribonucleotide precursors. Synthesis of dNTPs via this pathway is reported to occur in the cytosol, with dNTPs re-localizing to the nucleus through diffusion and to the mitochondria via transport mechanisms. The RR enzyme primarily exists as a heterodimeric tetramer of RRM1 and RRM2, the primary catalytic subunit. While RRM1 expression remains relatively constant in cycling cells, RRM2 is regulated in a cell cycle-specific manner and is degraded upon exit from mitosis and absent in quiescent cells. Given this, the RRM2 subunit is the primary regulator of RR enzymatic activity. RRM1 can alternatively associate with the p53R2 subunit in substitution for RRM2. Unlike RRM2, p53R2 is present in quiescent cells and as such associates with RRM1 under this context to provide dNTPs for mitochondrial DNA (mtDNA) replication in cells which are not actively dividing. Additionally, the expression of p53R2 is induced by the DNA damage response proteins p53 and p73, stabilized by ATM-mediated phosphorylation and is reported to be involved in the supply of dNTPs by RR for mtDNA replication and DNA damage repair. The role of RRM1 in the synthesis of dNTPs is also manifested as upregulation in cases of acquired resistance to gemcitabine and hydroxyurea, which directly inhibit the RR holoenzyme.

In the context of DNA damage response and repair, stable overexpression of RRM1 in lung adenocarcinoma cells results in an increased fraction of cells arrested in G2/M. This coincides with increased expression of the GADD45 G2 checkpoint protein, which is thought to mediate this arrest. In response to DNA-damaging agents, overexpression of RRM1 promotes the efficient repair of DNA damage. Additionally, RRM1-overexpressing cells have an increased level of apoptosis. When subjected to a murine chemical carcinogenesis protocol, mice transgenic for RRM1 (tg+) have significantly reduced lung tumor formation, and splenocytes derived from tg+ mice repair hydrogen peroxide-induced DNA damage more efficiently than those from tg- littermates. While the exact mechanism by which RRM1 contributes to DNA damage response and repair is unclear, it may involve its association with the p53R2 RR subunit. This subunit is induced by the DNA damage responsive p53 and p73 transcription factors, is stabilized by ATM-mediated phosphorylation and can substitute for RRM2 in the RR complex. Current data indicates a role for p53R2 in providing dNTPs during DNA damage repair and may explain the involvement of RRM1 in DNA damage response and repair.

With regard to tumor suppression, overexpression of RRM1 in human and mouse lung cancer cell lines, as well as ras-transformed mouse fibroblasts, inhibits metastasis, tumorigenicity and motility. It also suppresses metastasis formation and increases survival in a syngeneic murine lung cancer model. Evidence points to reduced phosphorylation of focal adhesion kinase (FAK) and increased PTEN expression as mediators of these effects. Mice transgenic for RRM1 subjected to a murine chemical carcinogenesis protocol display significantly decreased lung tumor development and survive significantly longer than tg-littermates. Additionally, multiple clinical studies show correlations between increased intratumoral RRM1 expression in surgically resected tumor tissue and increased survival (NSCLC, pancreatic cancer, bladder cancer). As previously noted, reduced FAK phosphorylation and increased PTEN expression are thought to be the primary mediators of the tumor suppressor function of RRM1; however, the ability of RRM1 to contribute to DNA damage response and repair may also play a role.

Homology

The RRM1 protein is highly conserved from human to many lower organisms. Human RRM1 protein shares 99.7% similarity and 97.6% identity with mouse (M. musculus) RRM1 and 88.2% similarity and 69.1% identity with that of fission yeast (S. pombe). It does not harbor significant homology to other human proteins.

Mutations

Germline

At this point, there are no described germline mutations within the RRM1 gene. There are a total of 272 reported single nucleotide polymorphisms (SNPs) for the RRM1 gene. Nine are in the coding region of RRM1, and four result in amino acid alterations which consist of: G249A, A768C, T821G and T2565C. We
sequenced the genomic region of RRM1 and deposited this data in GenBank (AF107045), which provided for the reference for some of the reported SNPs. In addition, we described SNPs in the RRM1 promoter region that have a substantial effect on in vitro reporter gene transcription.

**Somatic**

RRM1 resides at 11p15.5, a frequent region of allele loss in cancer. Additionally, amplification of RRM1 has been observed in acquired resistance to gemcitabine and hydroxyurea.

### Implicated in

**Non-small cell lung cancer (NSCLC)**

**Note**

Overexpression of RRM1 in human and mouse lung cancer cell lines inhibits motility in cell culture and decreases metastasis and tumorigenicity in a syngenic mouse lung cancer model. Increased survival is also noted upon RRM1 overexpression in the same model. Mice transgenic for RRM1 subjected to a chemical carcinogenesis protocol display significantly decreased lung tumor development and survive significantly longer than tg- littermates.

Since gemcitabine works in part through the inhibition of ribonucleotide reductase, RRM1 is a key molecule involved in the therapeutic response. Multiple NSCLC clinical studies demonstrate that while decreased intratumoral RRM1 is indicative of sensitivity to gemcitabine, elevated levels are predictive of poor response to gemcitabine. In NSCLC cell lines, depletion of RRM1 results in sensitivity to gemcitabine, and vice versa increased RRM1 expression results in resistance to gemcitabine. Gemcitabine-resistant NSCLC cells display upregulation of RRM1, and depletion of RRM1 in these cells restores gemcitabine sensitivity. Additionally, a 2464G>A RRM1 polymorphism was reported to be associated with gemcitabine sensitivity in cancer cell lines of various origins, while NSCLC patients harboring a combination of the RR37AC and RR524CT promoter polymorphisms, the haplotype associated with decreased promoter activity, show greater response to gemcitabine.

**Disease**

NSCLC is the most frequently diagnosed type of lung cancer and refers to malignant neoplasms of the lung which are histologically distinct from small cell lung cancer. NSCLC cases are classified as adenocarcinoma, large cell carcinoma or squamous (epidermoid) carcinoma. The National Cancer Institute (NCI) estimates that there were 215,020 new cases of lung cancer and 161,840 deaths in 2008.

**Prognosis**

Loss of the chromosomal region harboring RRM1 in NSCLC is associated with increased metastatic spread and decreased overall survival (OS) in patients with stage I or stage II disease. RRM1 mRNA derived from surgically resected NSCLC tumors indicates that high levels of RRM1 expression are associated with longer survival. This result was confirmed in a study where an elevated level of intratumoral RRM1 protein was prognostic of favorable survival. In addition to RRM1 expression, promoter polymorphisms at positions -37 and -524 (RR37AC and RR524CT), which regulate RRM1 expression in in vitro models, are associated with survival.

**Cytogenetics**

The RRM1 gene resides at 11p15.5, a region frequently associated with allele loss in NSCLC. This is exemplified in primary lung cancer tissue as well as lung cancer cell lines. The region of minimal allele loss in NSCLC is mapped to a 310 kb region which contains the complete coding sequence for the RRM1 and SSA/Ro52 genes. Of these two genes, RRM1 is identified as the putative tumor suppressor within this region of LOH.

**Pancreatic cancer**

**Note**

Increased RRM1 expression is indicative of resistance to gemcitabine in pancreatic cancer. RRM1 is the most highly upregulated gene observed in the 81-fold gemcitabine-resistant MiaPaCa2 derived cell line MiaPaCa2-RG, and siRNA depletion of RRM1 expression in this resistant cell line restores gemcitabine sensitivity. During the acquisition of gemcitabine resistance in pancreatic cell lines, progressive upregulation of RRM1 is observed.

**Disease**

The term pancreatic cancer refers to malignant neoplasms of the pancreas. Tumors of the pancreas can originate from either exocrine or endocrine pancreatic cells, with exocrine pancreatic cancer being the predominant form. The majority of exocrine pancreatic cancers are adenocarcinomas. The NCI estimates that there will be 42,470 new cases of pancreatic cancer and 35,240 deaths in 2009.

**Biliary tract cancer**

**Note**

Depletion of RRM1 expression via siRNA in the G-415 biliary tract carcinoma cell line increases sensitivity to gemcitabine and enhances gemcitabine-induced apoptosis. Tumor tissue derived from biliary tract carcinoma patients treated with gemcitabine shows a tendency toward elevated RRM1 expression associating with progressive disease (PD) and decreased RRM1 expression associating with partial response (PR), although these tendencies were not statistically significant. These results suggest that RRM1 expression may be a useful tool for predicting response to gemcitabine in patients with cancer of the biliary tract.
Disease

Biliary tract cancer refers to malignant neoplasms of the gallbladder and/or bile duct. Cancers of the bile duct are referred to as cholangiocarcinomas, while other cancers of the biliary system include gallbladder cancer and cancer of the ampulla of Vater. Biliary tract malignancies are relatively rare.

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