Gene Section
Review

DUSP26 (dual specificity phosphatase 26)

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Abstract

The DUSP26 gene encodes for an atypical dual specificity phosphatase commonly referred to as Dusp26 or MPK8. Although its physiological role is poorly understood, different substrates have been reported to be dephosphorylated by Dusp26, including p53, Kif3, Erk and p38. In this report we summarize the current knowledge on DUSP26 gene, its transcripts, the encoded protein and its function in normal and tumorous tissues. Notably, the phosphatase is overexpressed in neuroblastoma and ATC cells, where it promotes chemoresistance by inhibiting the p53 and the p38 proteins, respectively. Dusp26 represents a promising novel therapeutic target to be integrated with others and with conventional medicine, to improve survival outcome in patients and to reduce toxicity.

Keywords
DUSP26, dual-specificity phosphatase, MKP, phosphopeptides, MAPK

Identity

Other names: MKP-8, LDP-4, NEAP, SKRP3, DUSP24, NATA1

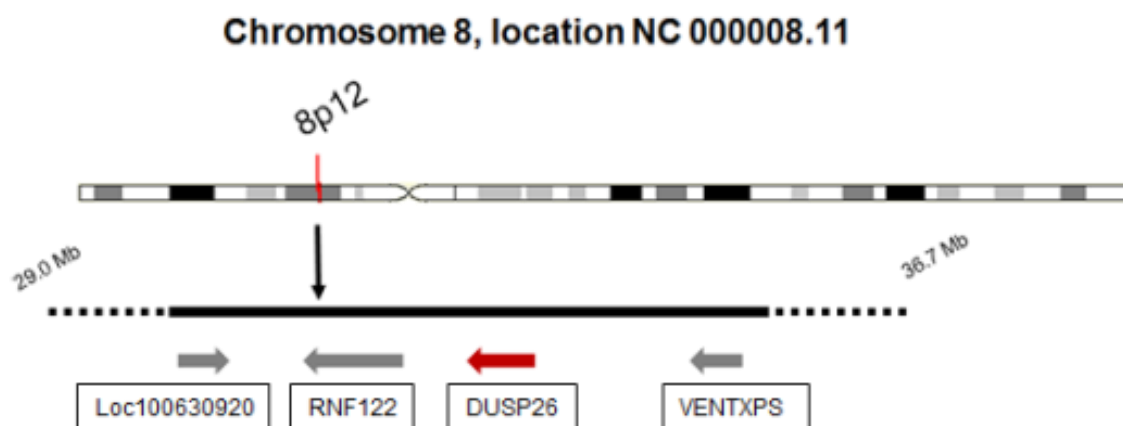**HGNC (Hugo):** DUSP26

Location: See Figure 1.

Figure 1: Chromosomal location of human DUSP26 gene, according to NCBI. DUSP26 gene is located on Chr 8, starting at 33600028 and ending at 33591330. The gene orientation is on the minus strand.
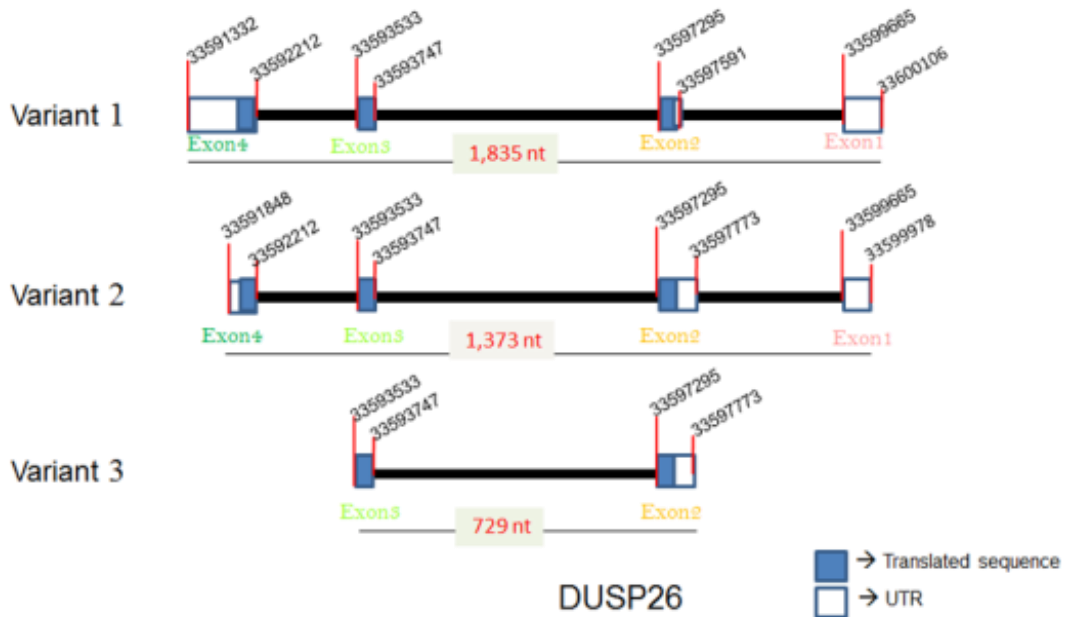
Figure 2: Human DUSP26 gene is located on chromosome 8. According to Ensemble (release of april 2015) the longest transcript is 1835 bases long and it consists of four exons (represented as boxes) and 3 introns (represented as lines). Two shorter isoforms have been reported, 1373 and 729 nucleotides long, respectively.

DNA/RNA

Description

The human DUSP26 gene is located on chromosome 8p12, starting at 33600028 and ending at 33591330 and comprises 4 exons (NCBI, 2015)

Transcription

According to Ensemble, three splicing variants have been reported for Dusp26 transcripts. The longest isoform comprises four exons and is considered the canonical sequence.

The second isoform also comprises four exons, and gives rise to a protein of the same length (211 aa), however it has shorter regulative sequences both at 3' and 5'. The third variant instead, consists of two exons and transcribes for a shorter protein, lacking 70 aminoacids at the C-terminus. In uniprot database this third variant is annotated in a different entry (E5RHD0), while a shorter isoform lacking the first 125 aminoacids is reported, which is not considered in ensemble.

Pseudogene

None

Figure 3: A) Schematic cartoon representing Dusp26 protein. The central Dusp26 phosphatase domain (residues 61-186), is surrounded by two N-terminal alpha-helices (helix alpha 1 and helix alpha 2) and a C-terminal helix (helix alpha 9) (Lokareddy et al, 2013). B) Ribbon depiction of the three dimensional structure of the catalytic domain of Dusp26 (aa 60-211) (adapted from PDB entry 4B04, (Won et al, 2013))
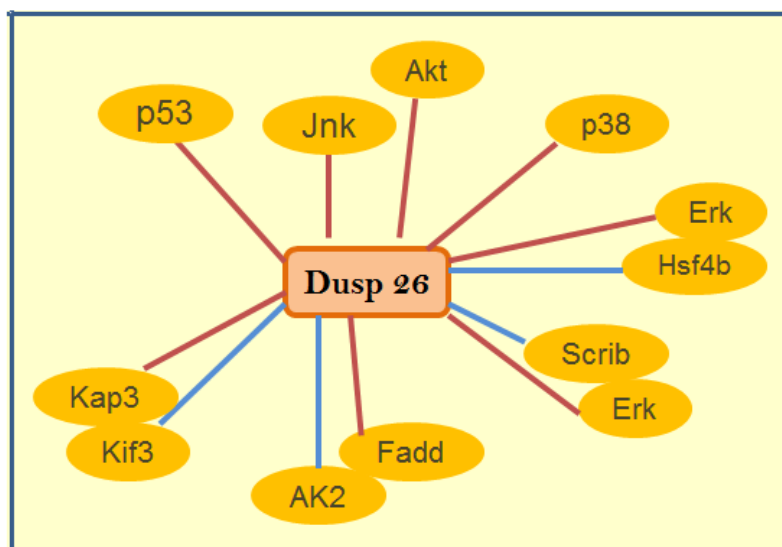
Figure 4: Graphical representation of Dusp26 interactors and substrates. Brown edges indicates phosphatase activity on the linked proteins, while blu edges link Dusp26 to scaffolding interactors. Dusp26 is a dual specificity phosphatase, and its molecular function is to dephosphorylate pS/pT or pY residues on the surface of its substrates. However, the physiological role of Dusp26 is still not completely elucidated, and it may be tissue-dependent (Patterson et al, 2010). Knockdown mice do not show severe malformations, presumably due to redundancy within the DSP family, but failure to thrive was reported, associated with decreased body weight, decreased circulating LDL, proteins and magnesium levels (http://www.mousephenotype.org/). In cultured PC12 cells (an adrenal-gland derived cell line that can differentiate into neuron-like cells) Dusp26 overexpression was shown to impair NGF-induced neuronal differentiation and to suppress neurite outgrowth (Wang et al, 2006). This is relevant when considering that Dusp26 was shown to be overexpressed in many human neuroblastoma cell lines and in primary tissue samples (Shang et al, 2010). However, in MCF10A epithelial cells the overexpression of the phosphatase resulted in reduced colony formation (Patterson et al, 2010)

Protein

Description

Dusp26 protein consists of 211 aminoacids with a predicted molecular mass of 23.945 kDa.

The phosphatase is an 'atypical DUSP' able to dephoshorylate both phosphotyrosine and phosphothreonine/phosphoserine residues, thanks to the 126 aminoacids DSP catalytic domain (Vasudevan et al, 2005).

This domain consists of five beta -sheets sandwiched between six alpha -helices (alpha 3-8), with a shallow catalytic site pocket. As shown in the diagram, the catalytic domain is surrounded by two N-terminal alpha -helices (alpha 1 and alpha 2) and one C-terminal helix (alpha 9) (Lokareddy et al, 2013).

The central core contains a PTP signature motif with the consensus Cys-(X)5-Arg-(Ser/Thr) conserved among the PTP class I members, where Cys152 and Arg158 form, together with Asp120, the catalytic triad.

The catalytic residue Cys152 which primes the reaction, is buried 7 ÅA below the enzyme surface, at the bottom of the catalytic site pocket (Lokareddy et al, 2013; Won, et al., 2013).

Dusp26 also presents an "AYLM" motif that is typical of MKPs, but lacks the CDC25 homology domain (Patterson et al, 2009; Vasudevan et al, 2005).

Expression

Dusp26 is expressed in the brain, heart, skeletal muscle, adrenal gland and spinal cord (Hu and Mivechi, 2006; Takagaki et al, 2007; Vasudevan et al, 2005; Wang et al, 2006). It is also expressed at lower levels in the testis and thyroid gland (Wang et al, 2006)

Localisation

Dusp26 localizes to the nucleus and the cytoplasm (Takagaki et al, 2007; Vasudevan et al, 2005; Wang et al, 2006)

Function

At the molecular level, several Dusp26 substrates have been reported and we will briefly summarize them. Dusp26 was reported to dephosphorylate MAPKs such as p38, Erk and JNK, as well as Akt kinase,and to regulate their activity (Hu and Mivechi, 2006; Vasudevan et al, 2005; Wang et al, 2006; Wang et al, 2008; Yu et al, 2007). However, other authors have reported no effect of Dusp26 on MAPK, which suggests that this activity might not be the major function of the phosphatase, or that it might behave differently in different cell types (Patterson et al, 2010). Notably Hsf4b and Scrib have been proposed as regulators to bridge the protein with MAPK substrates (Hu and Mivechi, 2006; Sacco et al, 2014). A similar role was proposed for the Adenylate Kinase (AK2), which was shown

to form a complex with Dusp26 and to stimulate its phosphatase activity toward Fas-associated protein with death domain (FADD),although the molecular mechanism of this activation is unknown (Kim et al, 2014).

However, the authors specify that this complex does not modulate apoptosis and that Fadd phosphorylation level is irrelevant to cell death, while it is involved in the control of cell proliferation.

The Kif3a subunit of the KIF3 protein-motor complex might be another scaffold subunit that leads the phosphatase to act on Kap3(Tanuma et al, 2009). It is not unfrequent that the catalityc activity of enzymes is increased upon binding to interactors that function as coactivators, and the organization of complexes could be a refined mechanism to control the specificity.

Notably, the structural comparison of Dusp26 with other Dusps reveals two gaps nearby the catalytic domain which could be targeted for the binding of physiological interactors or allosteric regulators (Won et al, 2013).

Dusp26 was also reported to directly bind and dephosphorylate Ser20 and Ser37 of the tumour suppressor p53, both in vitro and in vivo (Shang et al, 2010).

As a result of its function as MAPKs, KIF3 and p53 regulator, modulation of its activity and/or its expression has been proposed, as well as that of other atypical Dusps, as a promising anti-cancer therapy (Nunes-Xavier et al, 2011; Song et al, 2009).

The challenge for the future infact, is to complement various therapeutical targets, to treat patients with specific and more effective therapies. A current priority is the elucidation of the molecular basis for the insorgece of acquired drug resistance during therapy, and for the selection of apoptosis-resistant cells.

Although more than 50% of all human cancers have p53 deletion or mutation, neuroblastoma (NB) is known to maintain functional p53, but to defect in apoptotic pathways.

It was shown that Dusp26 has an important role in the chemoresistance of NB cancer cells, and inhibits doxorubicin- and genotoxic-stress-induced apoptosis by dephosphorylation and inactivation of p53.

In Dusp26 knockdown cells there is a significant enhancement of apoptosis and a concomitant iperphosphorylation of p53, while the overexpression of the phosphatase promotes cell resistance (Shang et al, 2010). Althought Dusp26 is

overexpressed in neuroblastoma cells, as described

below, no specific drug has been developed yet to selectively target Dusp26 (Song et al, 2009). However it is reasonable to think that interfering Dusp26 dephosphorylation of p53 would promote NB cells apoptosis and help chemosensitivity (Shang et al, 2010).

Similarly, in anaplastic tyroid cancer cells (ATC), the phosphatase downregulates apoptosis by inhibiting p38 MAPK (Yu et al, 2007).

In PC12 cells, however, low levels of the phosphatase are required for differentation, while Dusp26 overexpression down-regulates the PI3K/Akt pathway.

In these conditions, NGF-induced phosphorylation of mTOR on Ser2448, the Akt specific targeted serine, is decreased, and PC12 cells are more sensible to cisplatin-induced apoptotic stimuli (Wang et al, 2006).

Beyond regulating cell differentiation and apoptosis in these cell systems, Dusp26 was also hypothesized to control cell transformation in other human malignancies, by exerting a tumour-suppressor activity enhancing cell-cell adhesion. Dephosphorylation of the Kap3 subunit of the KIF3 microtubule-dependent protein motor infact, results, by unknown mechanism, to N-cadherin/beta-catenin colocalization in membrane ruffles and in sites of cell contacts (Tanuma et al, 2009).

These findings suggest that the final effect of the phosphatase depends on tissue-specific substrates and a fine characterization of its fuctional role in different cancer cells is necessary to develop selective therapies.

While the catalytic core of the atypical Dusps is well conserved among the members of the family, the binding of ligands to more specific N and C terminal regions (Sacco et al, 2014) allows for the design of selective inhibitors.

Homology

Dusp26 is a member of the dual specificity family of protein phosphatases.

It shares the highest homology with Dusp13, Dusp 27 and Dusp3/VHR protein phosphatases (Patterson et al, 2009; Pavic et al, 2015; Takagaki et al, 2007; Vasudevan et al, 2005; Won et al, 2013).

The human Dusp26 protein is highly conserved within Mammalia, and shows 100%, 98%, 97% and 94% of identity with chimpanzee, rat, mouse and pig orthologs, respectively (percentages according to Blast bl2seq).

Dusp26 also shows high similarity with chicken, chamaleon, western clawed frog, Japanese ricefish and zebrafish homologous proteins.
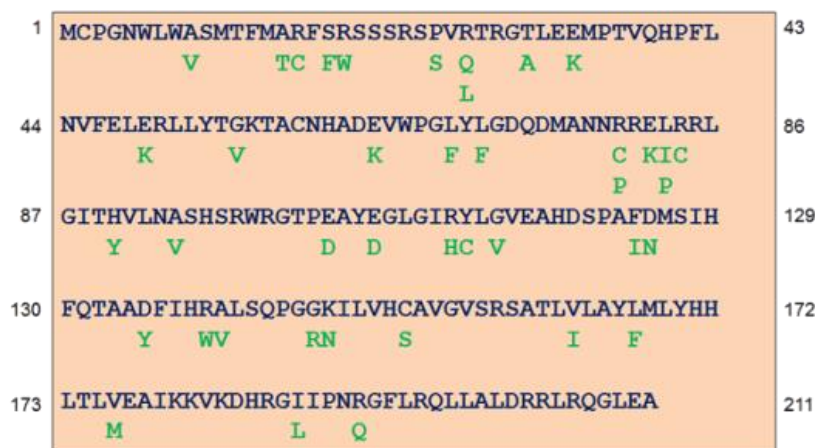
Figure 5: Missense mutation of Dusp26 (reported in Cosmic database). 41 missense mutation are reported in Cosmic. Two different missense substitutions were found in position 441 of the cDNA (441G>C; 441G>T) both leading to the mutation of the Lysine 147 in to an Asparagine. Substitution of Cysteine 152 to Serine instead is described elsewhere.

Mutations

Somatic

Several point mutations of Dusp26 coding region are reported in Cosmic database (Forbes et al, 2015). The analysis of 19779 samples led to the identification of 41 missense mutations, 16 synonymous and 2 nonsense mutations (Forbes et al, 2015) (april 2015 release).

None of them are associated with a specific phenotype.

The mutation C152S instead, inactivates the phosphatase function of the protein (Vasudevan et al, 2005; Wang et al, 2006).

Amplification of genomic copy number of DUSP26 gene, overexpression of its transcript, as well as downregulation, have all been reported to be related to human cancers (see also below).

Actually, the amplification of the genomic copy number of DUSP26 was reported in Anaplastic Thyroid Cancer cells, and the consequential overexpression of the transcript was determined by RT-PCR (Yu et al, 2007).

Overexpression of DUSP26 was determined at RNA and protein level in neuroblastoma cell lines and in primary tumour samples(Shang et al, 2010). Downregulation of Dusp26 RNA was also observed in ovarian cancer, medulloblastoma and glioblastoma cell lines (Patterson et al, 2010)

Implicated in

Anaplastic Thyroid Cancer (ATC)

Note

The amplification of 8p11-12 region (containing the DUSP26 gene) has been observed in many types of cancer: breast, bladder, lung and ovarian cancer; however no target gene has been identified in those cases.

In Anaplastic Thyroid cancer instead, the overexpression of DUSP26 mRNA was confirmed in (Yu et al, 2007), and its oncogenic activity was dependent on its phosphatase activity on p38.

Disease

Thyroid carcinomas are classified in medullary, papillary, follicular and anaplastic. This last form is relatively rare, constituting to less than 2% of all thyroid cancers, but shows extremely aggressive behaviour and few effective treatment options, resulting in high mortality rates (Schiff et al, 2004).

Prognosis

Patients with ATC have a grave prognosis, with a mortality rate of almost 100% and a mean survival of few months (Schiff et al, 2004)

Neuroblastoma (NB)

Note

DUSP26 was overexpressed in some (but not all) neuroblastoma cell lines and in retinoblastoma and neuroepithelioma cell lines (Patterson et al, 2010; Shang et al, 2010; Vasudevan et al, 2005). Immunohistochemistry showed high DUSP26 expression in high-risk neuroblastoma samples compared with low risk groups (Shang et al, 2010). The phosphatase was shown to contribute to the resistance of neuroblastoma cells to doxorubicin-induced apoptosis, by dephosphorylating the tumour suppressor p53 (Shang et al, 2010).

Disease

Neuroblastoma is a tumour of the sympathetic nervous system and is the most common solid tumour in children (Brodeur et al, 2014). NB cells can be hyperdiploid or near triploid, with chromosomal aberration, rearrangements and gene amplfications (Brodeur, et al., 2014). Several genes have been reported to be activated in NB: MYCN, ODC1, ALK, TRK, PTPN11 (Brodeur, et al, 2014) and DUSP26 (Shang et al, 2010)

Prognosis

The clinical behaviour of this disease is highly heterogeneous, and spans from spontaneous regression, frequent in children under 18 months of age, to metastatic tumours with 40-50% survival rate (Brodeur and Bagatell, 2014).

Glioblastoma (GBM)

Note

Different forms of GBM display distinct molecular abnormalities, as reviewed in (Veliz et al, 2015) . Gene amplifications, deletions and/or overexpression have been reported, and, among them, loss of the dual specificity phosphatase PTEN is a very common feature (Veliz et al, 2015). Dusp26 mRNA expression is also downregulated in human glioblastoma cell lines and in primary tumour samples and its downregulation was associated with invasive phenotypes (Patterson et al, 2010; Prabhakar et al, 2014; Tanuma et al, 2009; Vasudevan et al, 2005).It has been proposed that Dusp26 enhances cell-cell adhesion in GBM by promoting N-cadherin/Beta-catenin localization at the plasma membrane (Tanuma et al, 2009).

The downregulation of Dusp26 mRNA has been observed also in ovarian cancer and medulloblastoma cell lines (Patterson et al, 2010)

Disease

Glioblastoma Multiforme (GBM)is a genetically heterogeneous group of brain tumours, which originates from glial cells (Urbanska et al, 2014). The 90% of GBM cases are primary tumours, developed from normal cells by multistep tumorigenesis, while 10% are secondary tumours developed from astrocytomas (Urbanska et al, 2014). The two types of tumours show no morphological differences, both presenting small cells with increased nuclear to cytoplasmic ratios, however secondary tumours grow slower and have a better prognosis (Urbanska et al, 2014).

Prognosis

Although metastases have been rarely reported in GBM, primary tumours are very aggressive, with overblown vascularization and resistance to conventional radiotherapy and chemotherapy, and with a 5-year survival rate of 5% (Veliz et al, 2015).

To be noted

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