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Gene Section
Review

NUSAP1 (nucleolar and spindle associated protein 1)

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Abstract

NUSAP1 (Nucleolar and Spindle Associated Protein 1) is a cell-cycle dependent protein highly expressed during mitosis. It has microtubule-binding and DNA-binding activity, which underlies its best-characterised function as a regulator of the mitotic apparatus in dividing cells. Deregulated abundance of NUSAP1 is associated with misassembly or misfunction of the mitotic spindle during mitosis, originating genetic instability in daughter cells. Roles in cell migration, invasion and metastases are also reported, as well as functional interactions with pro-oncogenic pathways. NUSAP1 is overexpressed in several cancer types and is regarded as a novel prognostic biomarker. Experimental down-regulation of NUSAP1 abundance often inhibited cell proliferation in several cancer contexts. NUSAP1 is also proposed as a therapeutic target, with the potential to improve the outcome of treatments in combination therapy for certain cancer types.

Keywords
NUSAP1, nucleolus, mitosis, microtubules, mitotic apparatus, chromosome segregation, genetic instability, cancer prognostic marker

Identity

Other names: FLJ13421, LNP, ANKT, NUSAP, SAPL, BM037, PRO0310p1, Q0310

HGNC (Hugo): NUSAP1

Location: 15q15.1

Location (base pair): Starts at 15: 41,332,694 and ends at 41,381,050 bp from pter (from NCBI)

DNA/RNA

Description

The NUSAP1 gene spans 60,257 bases located on chromosome 15, at cytogenetic bands 15q14 (according to HGNC), 15q15.1 (according to Entrez Gene), 15q15.1 (by Ensembl)

Transcription

Both the mouse Nusap1 and human NUSAP1 genes are selectively expressed in proliferating but not in differentiated cells. Murine Nusap1 was originally identified as a selectively expressed transcript in proliferating but not in differentiated mouse MC3T3E1 osteoblasts by differential display. The mouse cDNA full-length sequence contains a single ORF of 1,281 bp that encodes a protein of 427 aminoacids. Cloning of the human NUSAP1 cDNA and comparison with EST (expressed sequence tags) databases (https://www.ncbi.nlm.nih.gov/genbank/dbest) showed high conservation of NUSAP1 in vertebrates (Raemaekers et al., 2003). In particular the human NUSAP1 cDNA contains an ORF of 1323 bp encoding a protein of 441 aminoacids (source Uniprot).

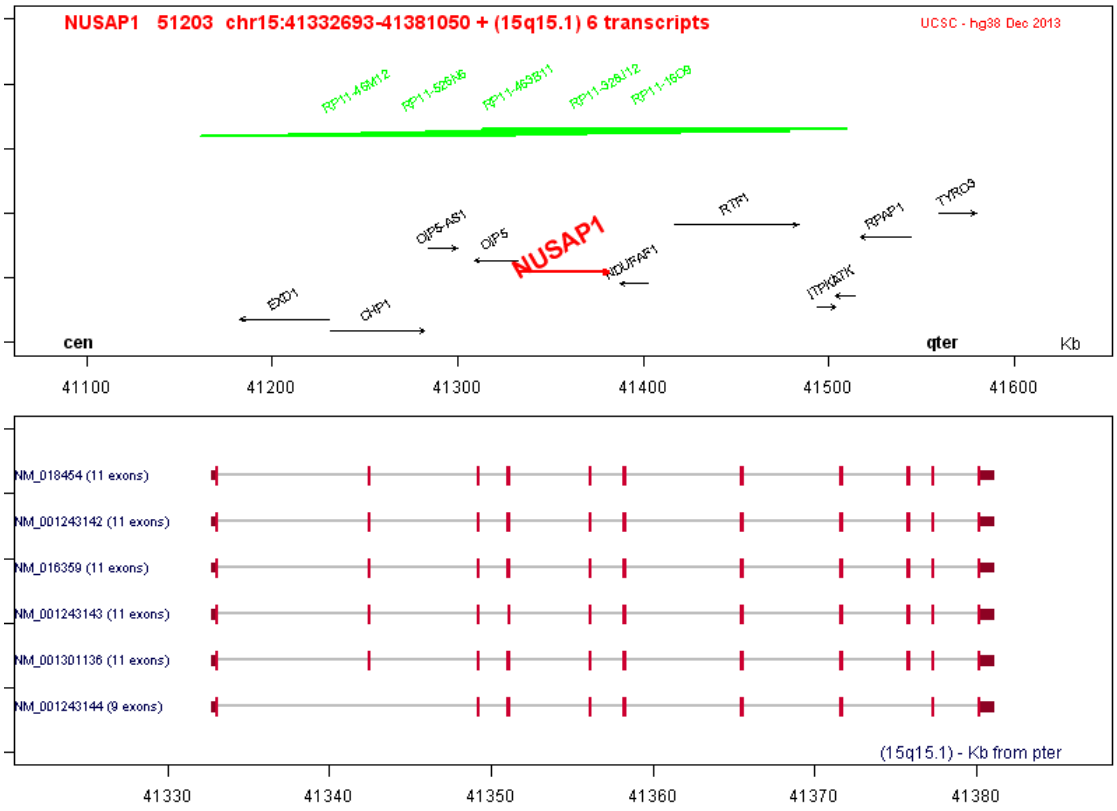

Figure 1. Genomic map

The intron/exon structure for human NUSAP1 is available at https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieved opt=Graphicslist_uids=51203. The NUSAP1 gene is overexpressed in several carcinoma types and in tumours of the central nervous system (see below). This is likely linked, at least in part, to transcriptional regulation by the cell cycle transcription factor E2F1 (Gulzar et al., 2013) and the MYC proto-oncogene (Hussain et al., 2008).

Protein

7 potential products may be generated from alternatively spliced isoforms (from UniProt/SwissProt), but isoform 1 (https://www.uniprot.org/uniprot/Q9BXS6#Q9BXS6-1) is regarded as the canonical protein. The human NUSAP1 protein (441 aminoacids) has a calculated molecular mass of 48.6 kDa (isoelectric point, 9.9). The apparent molecular mass is 55 kDa, and the difference between expected and observed may be accounted for, at least in part, by phosphorylation by cell cycle kinases.

Biological overview: NUSAP1 (Nuclear and Spindle Associated Protein) is a cell-cycle dependent protein and is the only product of the NUSAP1 gene, which is selectively expressed in proliferating cells (Raemaekers et al., 2003).

Both the mRNA and protein abundance peak in the G2 and M phases of the cell cycle and sharply decrease at mitotic exit.

NUSAP1 is a microtubule-associated protein (MAP) and has microtubule-bundling and stabilizing activity in vitro.

This underlies its main function as a regulator of the mitotic apparatus in dividing cells (Raemaekers et al., 2003; Ribbeck et al., 2006).

At mitotic onset, NUSAP1 localises to the spindle microtubules in the region that comes in contact with chromosomes, and stabilises their interaction, thus contributing to regulate chromosome segregation to daughter cells.

Deregulated abundance of NUSAP1 is associated with misassembly or misfunction of the mitotic spindle and contributes to generate genetic instability in daughter cells through faulty mitosis.

NUSAP1 also has roles in invasion (Gordon et al., 2017) and in functional interactions with pro-oncogenic pathways.

High levels of NUSAP1 are often associated with poor prognosis.

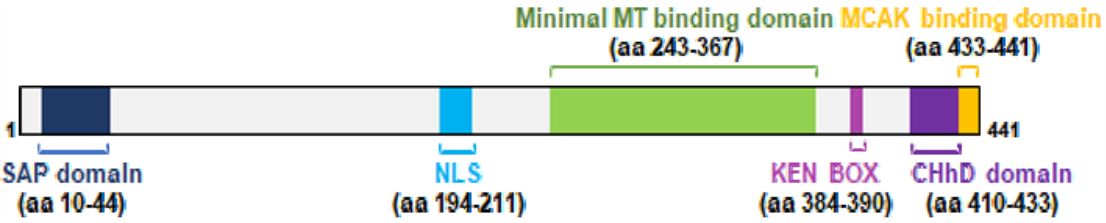

Figure 2. Schematic map showing relevant functional domains in the human NUSAP1 protein (details in the text)

Description

The human protein NUSAP1 contains several domains conserved in the mouse protein, first described by Raemaekers et al. (2003).

- a helix-extension-helix domain (aa 10-44), containing a potential SAP motif - the latter, named after three proteins that share it, i.e. SAF-A/B, Acinus and PIAS, is found in various nuclear proteins involved in transcription, DNA repair, and RNA processing. The SAP motif, which is essential for DNA binding in the SAF-A/B protein, is also thought to act as the DNA-binding motif in many nuclear proteins (https://www.ebi.ac.uk/interpro/entry/InterPro/IPR003034/), and is reported to confer DNA-interacting capacity to the NH2 terminal (N-ter) region of NUSAP1 (Verbakel et al., 2011),
- a bipartite nuclear localisation signal, NLS (aa 194-211) that can interact with KPNA1 and KPNB1 (importin-alpha and -beta),
- the microtubule-binding domain with a minimal essential core located between residues 243-367,
- a KEN box (aa 383-390), required for protein degradation (Li et al., 2007),
- a highly charged domain with a predicted helical structure, called the charged helical domain (ChHD; aa 410-433), in the COOH terminal (C-ter) region,
- the C-ter region containing the MCAK-binding domain (MCBD), (aa 433-441), responsible for direct binding to KIF2C (or MCAK, mitotic centromere-associated kinesin) and implicated in control of the depolymerisation activity of MCAK at the kinetochore region and, thus, for regulating the stabilisation of kinetochore-microtubules attachments during prometaphase and metaphase (Li et al., 2016).

Phosphorylation sites for various cell cycle kinases, including AURKB (Aurora-B) (Hussain et al., 2008; Ozlu et al., 2010), AURKA (Aurora-A) (Sardon et al., 2010), CDK1 (Chou et al., 2011) and ATM (Xie et al., 2011) have been experimentally identified.

Expression

The NUSAP1 gene is transcribed from late S to G2 phases of cell cycle, yielding highest protein abundance in M phase. At mitotic exit protein levels decrease via the proteasome/ ubiquitination system, involving the ubiquitin ligase APC/C/Cdh1 (Li et al., 2007), the major regulator of protein ubiquitin conjugation in late mitosis (Raemaekers et al., 2003).

After cytokinesis and in the next G1 phase, NUSAP1 is degraded after ubiquitination by the M-specific ubiquitin ligase APC/C (Anaphase-promoting complex /cyclosome) in complex with its activator Cdh1 (Li et al., 2007).

The protein is expressed in different proliferating tissues (e.g. bone marrow, lymph nodes, testis) and is overexpressed in different cancer types (see below).

Localisation

NUSAP1 localisation is cell cycle-dependent by immunofluorescence (IF) assays. When NUSAP1 first appears in interphase G2 cells, it localises within the nucleus and concentrates at nucleoli. Cell fractionation experiments confirmed that nuclear/nucleolar localisation (Raemaekers et al., 2003).

In mitosis, after nuclear envelope breakdown, soluble NUSAP1 is released in the cytoplasm and localises on microtubules in prometaphase, gradually concentrating at microtubule plus (+) ends near chromosomes until anaphase. In late anaphase, the soluble NUSAP1 fraction becomes barely detectable, and virtually the entire NUSAP1 pool associates to thick microtubules bundles around chromosomes, forming characteristic NUSAP1 'spikes' in telophase (Raemaekers et al., 2003).

Function

As a microtubule-associated protein, NUSAP1 plays key roles in mitosis. For clarity, we will separately summarise NUSAP1 roles in a) cell cycle progression; b) microtubules-associated activity; c) spindle formation and dynamics; d) DNA-binding activity.

a) Cell cycle progression

NUSAP1 is essential to proper cell cycle progression. Indeed, NUSAP1 depletion following RNA interference delays entry into mitosis and arrests cycling cells at the G2/M transition. Under this condition, dramatic mitotic defects are depicted by IF, e.g. abnormal spindles in prometaphase and altered chromosome condensation.

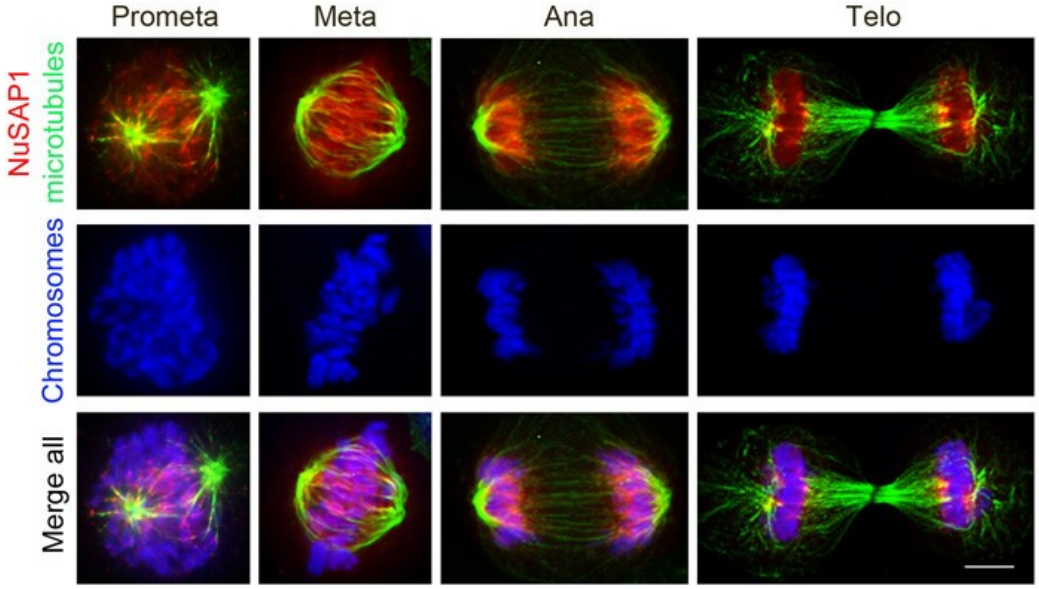

Figure 3. NUSAP1 localisation during mitotic progression in human HeLa cells. In prometaphase NUSAP1 (red) accumulates at the growing ends of microtubules, distal from the originating asters (green); during this stage microtubules establish contact with chromosomes (blue), which eventually align at the cell equator (metaphase). Chromosomes attached to NUSAP1-decorated microtubule ends initiate segregation in anaphase; later, in telophase, NUSAP1 is detectable in 'spikes' and in a diffuse pattern in the decondensing chromatin. An antibody against alpha-tubulin (green) stains the spindle microtubules; DAPI (blue) stains chromosomes (Image by Michela Damizia. The mitotic localisation of complexes containing NUSAP1 and Importin beta is shown in Di Francesco et al., 2018).

Mitotic cells that still proceeded towards chromosome segregation with disorganised spindles underwent abnormal anaphase and defective cytokinesis (Raemaekers et al., 2003). On the other hand, NUSAP1 overexpression also leads to mitotic arrest (Li et al., 2007) due to the formation of thick microtubule bundles that impair the dynamic activity of the spindle. These results indicate the critical importance of regulated levels of NUSAP1 protein to ensure proper cell division.

b) Regulation of microtubule activity

- NUSAP1 binds and stabilises microtubules

Microtubule sedimentation assays separate polymerised microtubules from free dimers of alpha/beta tubulin, and they are used to study microtubule-associated proteins (MAPs). In these assays, NUSAP1 binds directly to polymerised microtubules (Raemaekers et al., 2003). NUSAP1 can effectively cross-link microtubules into asters and thick fibers, an essential step in assembly of the mitotic apparatus. Moreover, NUSAP1 binding protects microtubules against depolymerisation and helps 'guiding' the microtubules towards the chromosomal DNA, an essential step prior to chromosome segregation (Ribbeck et al., 2006).

- Importins and the GTPase RAN regulate the activity of NUSAP1 on microtubules

NUSAP1 interacts with nuclear import receptors: importin alpha, which recognises the NLS; Importin beta and IPO7 (Importin 7) (the actual import vectors for NUSAP1), in both biochemical experiments (Ribbeck et al., 2006) and in intact cells (Di Francesco et al., 2018). In interphase, this interaction mediates NUSAP1 import in nuclei.

In vitro assays indicate that NUSAP1 functions are differentially affected by importin beta and importin 7.

Indeed, the presence of Importin beta alone impaired NUSAP1 function in the production of aster-like microtubule structures, while not affecting the production of long tubulin fibers; on the contrary, Importin 7 alone prevented NUSAP1 activity in generating long fibers and only aster structures were produced. RANGTP addition reversed the inhibitory effect exerted by either importin on NUSAP1 functions on microtubules, suggesting that the release of free NUSAP1 from Importin beta and -7 interactions is RANGTP-dependent (Ribbeck et al., 2006).

- Phosphorylation of NUSAP1 regulates its functions

NUSAP1 is phosphorylated by the major cell cycle-dependent kinase, CDK1, at threonine 300 and 338 in the microtubule-binding domain. In in vitro microtubule sedimentation assays, phosphorylated NUSAP1 (ph-NUSAP1) fails to bind microtubules, suggesting that CDK1-mediated phosphorylation negatively regulates NUSAP1 interaction with microtubules. During the cell cycle, ph-NUSAP1 is detectable in nucleoli in prophase; later, after nuclear envelope breakdown, it diffuses in the cytosol and around condensed chromosomes; finally, the signal disappears during progression from anaphase to telophase, (Chou et al., 2011).

NUSAP1 can also be phosphorylated by Aurora kinases. Aurora-A was shown to be able to phosphorylate NUSAP1 at Serine 240; although the functional significance of this phosphorylation remains unclear, it may have roles during microtubule assembly (Sardon et al., 2010). NUSAP1 was also identified in a proteomic screening for Aurora-B substrates; this phosphorylation putatively involves several serines or threonines in the N-ter region and is proposed to regulate NUSAP1 activity at the spindle midzone at cytokinesis (Ozlu et al., 2010).

c) Spindle formation and dynamics

- NUSAP1 regulates spindle formation

Xenopus egg extract provide an informative model system to study spindle assembly and function. They derive from unfertilised eggs arrested in metaphase II of meiosis and contain all maternal factors which only need be organised and activated at fertilisation. Immunodepletion experiments show that NUSAP1 is essential for spindle formation. Indeed, in NUSAP1-depleted extracts, abnormal spindles formed, which showed a low microtubule density and a distorted bipolar organisation (Ribbeck et al., 2006). Instead, excess NUSAP1 blocked the onset of spindle formation. When NUSAP1 was added after the spindle had already started to form, the microtubules became strongly bundled into prominent fibers, resulting in a distorted configuration. Thus, the amount of NUSAP1 regulates the balance of microtubules in the spindle (Ribbeck et al., 2006).

-NUSAP1 regulates spindle dynamics

NUSAP1 interacts with MCAK, a kinesin with microtubule-depolymerizing activity, required to correct any misattachments between microtubules and kinetochores occurring during spindle formation. Li and colleagues demonstrated that NUSAP1 regulates the depolymerizing activity of MCAK, assigning to NUSAP1 a novel role in microtubule dynamics (Li et al., 2016). It has also been discovered that NUSAP1 also contributes to orchestrate the alignment and orientation of chromosomes in the metaphase plate by regulating the activity of Kid, a chromokinesin that guides the anti-poleward movements of chromosomes: thanks to its ability to use and hydrolyze ATP, Kid generates a force along the spindle defined the 'ejection force'. NUSAP1 binds Kid and facilitates its association with microtubules, thus governing the pole ejection force generated by Kid (Li et al., 2016).

- NUSAP1 interacts with the nucleoporin and E3 SUMO ligase RANBP2

Mass spectrometry-based analysis of NUSAP1 interacting partners revealed a novel interaction with RANBP2, a nucleoporin endowed with SUMO E3 ligase activity, and its partners, the RANGTP hydrolysis-activating enzyme RANGAP1 and the SUMO conjugating enzyme UBE2I (Ubc9) (Mills et al., 2017). Proximity ligation assays (PLA) localised the interaction on microtubules (Mills et al., 2017).

In summary, NUSAP1 controls the mitotic apparatus in several ways: it regulates microtubule stability both directly, via its own microtubule-bundling activity, and indirectly, by regulating the MCAK microtubule-depolymerizing enzyme; it also regulates Kid-generated pole ejection forces in the spindle. These functions converge to regulate proper chromosome segregation during mitosis.

d) DNA-binding activity

NUSAP1 can also interact with the DNA via the SAP domain in the N-ter region (Verbakel et al., 2011). In vitro, the SAP domain preferentially binds double-stranded DNA. During mitosis, wild-type but not SAP-deleted NUSAP1 (NuSAPΔSAP) localises not only to chromosome proximal microtubules, but also to chromosome arms, indicating that the SAP domain mediates the association of NUSAP1 with chromatin in early mitosis. In interphase nuclei, both WT and NuSAPΔSAP are found in nucleoli, but only wild-type NUSAP1 also assumes its canonical localisation to the periphery of the nucleoplasm, where active chromatin domains reside; possible roles of NUSAP1 in transcription or in the DNA damage response have therefore been suggested, as found for other SAP domain-containing proteins (Aravidin and Koonin 2000).

Homology

The NUSAP1 gene has conserved orthologues in chimpanzee, macaque, dog, cat, cow, horse, pig, opossum, mouse, rat, chicken, zebrafish, drosophila and frog (sources NCBI and HGCN, HCOP homology predictions).

Mutations

Fusion genes: Data from Atlas, Mitelman, Cosmic Fusion, Fusion Cancer, and TCGA fusion databases

CD74 (5q32) / NUSAP1 (15q15.1)

NUSAP1 (15q15.1) / RRNAD1 (1q23.1) (Klijn et al., 2015)

NUSAP1 (15q15.1) / ZNF445 (3p21.31) (Wang et al., 2015)

OIP5 (15q15.1) / NUSAP1 (15q15.1) (Yoshihara et al., 2015)

Expected fusion products by conceptual translation of open reading frames generated by translocations:

ITPKA/NUSAP1 fusion, from translocation t(15;15)(q15;q15) (Gao et al., 2018)

MGA /NUSAP1 fusion, from translocation t(15;15)(q15;q15) (Gao et al., 2018)

Decipher database

The region of chromosome 15 containing, among others, the NUSAP1 locus is involved in sporadic

cases of deletions or duplications associated with complex phenotypes; of those, the most selective for NUSAP1 are:
heterozygous deletion 15:41285354-45826511 (removing 4,5 Mbp), associated with facial and intellectual disabilities;
heterozygous deletion 15:41285354-45826511 (removing 6,9 Mbp), associated with microcephaly, intellectual disabilities and other traits.
Overall, these phenotypes are consistent with the notion that mutation or deletion of mitotic genes, when occurring early in development, often cause failed development of the central nervous system (Lang and Gershon, 2018; Degrassi et al., 2019).

Implicated in

Top note
Both NUSAP1 mRNA transcript and protein product are overexpressed in several types of cancer compared to their healthy tissue counterpart, as summarised below. In many instances, down-regulating NUSAP1 expression inhibits cancer cell proliferation, which formally defines NUSAP1 as a proto-oncogene. Consistent with the mitotic roles of NUSAP1 illustrated above, these antiproliferative effects are often exerted by depressing the mitosis-promoting activity of NUSAP1 and arresting the cell cycle with a G2/M content, generally followed by cell death. NUSAP1 also influences cell migration, invasion, and metastasis-inducing capacity by affecting the cytoskeleton and cytoskeletal signaling. Many studies converge to indicate that high NUSAP1 levels are prognostic of poor outcomes and suggest that assessing NUSAP1 levels in cancer tissues of individual patients might help to improve the outcome of therapeutic treatments in a precision medicine perspective.

Breast cancer

NUSAP1 is involved in the onset and progression of breast cancer. Chen et al. (2015) investigated NUSAP1 expression in breast cancer by constructing two sets of tissue microarrays (TMAs) that contained 450 stage-I to -III primary breast cancer tissues. By immunohistochemistry, NUSAP1 was found to be specifically overexpressed in triple-negative breast cancer (TNBC). Consistent with this, a Kaplan-Meier plot, constructed to evaluate the prognostic value of NUSAP1, showed that high NUSAP1 expression correlated with poor disease-free survival (DFS) specifically in TNBC patients. By stratifying for the status of both NUSAP1 and BRCA1, low BRCA1 expression was associated with DSF in TNBC, indicating that combining both markers can provide additional prognostic information for TNBC (Chen et al., 2015).

Zhang et al. (2018) further identified NUSAP1 in a screening for Invasive Breast Cancer (IBC)-related genes, in which NUSAP1 was found to have higher expression compared to normal tissues. Similar results were obtained from the MCF7 breast cancer cell line. In functional assays, downregulation of NUSAP1 led to reduced proliferation in MCF7 cells, associated with downregulation of both the mitotic kinase CDK1 and the mitotic spindle-stabilizing factor DLGAP5 (HURP), both required for mitotic progression. Cell migration and invasion also decreased in NUSAP1-interfered compared with untreated BC cells. The development of innovative cancer therapies based on the gene expression profile is key to overcome chemotherapy resistance in IBC. Remarkably, in that respect, NUSAP1-interfered MCF7 cells show increased susceptibility to Epirubicine (E-ADM) treatment, enhancing E-ADM-induced apoptosis (Zhang et al., 2018). Thus, combination therapies including NUSAP1 targeting and traditional compounds, e.g. Epirubicine, might be envisaged in IBC treatment.

Cervical cancer

NUSAP1 is upregulated in cervical cancer (CC) cells and tissues. Immunohistochemical assay on 233 cervical cancer samples showed moderate (49%) or strong (27%) levels of NUSAP1 protein. Moreover, high expression of NUSAP1 positively correlates with the lymph node metastasis and poor clinical outcome. The possible role of NUSAP1 in CC metastasis was studied in Matrigel-coated transwell assays using both NUSAP1 stably overexpressing, and knocked down, HeLa and SiHa CC cell lines. NUSAP1 upregulation yielded an increase, and downregulation a decrease, in cell migration and invasion. In vivo experiments in models for lung colonisation and popliteal lymph node metastases, also demonstrated that NUSAP1 overexpression enhances the metastatic power of NUSAP1-overexpressing SiHa cells in lung and in lymph node in mice 45 days after injection. Furthermore, in combined Western blot (WB) and IF assays NUSAP1 overexpression was associated with an increased level of mesenchymal markers and a decrease of epithelial markers, suggesting a role for NUSAP1 in EMT transition. These findings implicate NUSAP1 in CC metastases. In addition, cells with high NUSAP1 expression also showed an enrichment in stem cells-associated genes, suggesting a role of NUSAP1 in maintenance of cancer stem cell properties in cervical cancer.
NUSAP1 upregulation is associated with CTNNB1 (beta-catenin) translocation into the nucleus, which enhances the transcriptional activity of TCF4 (a member of TCF/LEF transcription factors) on target genes.

Actually, NUSAP1 overexpression enhances RANBP2 mediated-SUMOylation of TCF4. RANBP2 knockdown, like NUSAP1 knockdown, inhibits Wnt/beta-catenin signaling and TCF transcriptional activity (Li et al., 2019). CC treatment with XAV-939, an inhibitor of the Wnt/beta-catenin pathway, abrogated NUSAP1-dependent cancer stem cell metastasis. Based on these data, NUSAP1 is thought to promote CC metastatic progression via activation of beta-catenin signaling and downstream transcriptional programmes. The authors suggest that the Wnt/beta-catenin inhibitor XAV-939 might improve the outcome of canonical drugs in NUSAP1-overexpressing CC patients.

Prostate cancer

NUSAP1 was identified as a prognostic biomarker of prostate cancer: gene expression profiling of 98 prostate tumours vs. 52 benign adjacent prostate tissues identified NUSAP1 as an overexpressed gene in prostate tumours, associated with recurrence after prostatectomy. Post-transcriptional silencing of the NUSAP1 gene inhibits the ability of PC3 to invade and proliferate in vitro. Functional analysis using Significance Analysis of Microarrays, cDNA microarray, and RT-qPCR, showed a positive correlation between expression of the E2F1 transcription factor and NUSAP1 transcript, and, on the contrary, a negative correlation between the retinoblastoma gene product pRb and NUSAP1 in prostate cancer cell lines, supporting the possibility that the pRb-E2F1 pathway is involved in regulation of NUSAP1 transcript levels (Gulzar et al., 2013). This hypothesis was confirmed by Gordon et al. (2015) in a study demonstrating that NUSAP1 transcription was upregulated after knockdown of RB1, implicating the pRB1/E2F1 axis in NUSAP1 regulation in prostate cancer cells. In turn, upregulation of NUSAP1 abundance supports prostate cancer progression by increasing proliferation and invasion of prostate cancer cells (Gordon et al., 2017). The role of NUSAP1 in invasion, migration, and metastatic potential of prostate cancer cells, has been related to modulation of an important effector of transforming growth factor beta 1 (TGFB1), i.e. RFLNB (family with sequence similarity 101 member B FAM101B), which is involved in the epithelial to mesenchymal transition (Gordon et al., 2017).

Bladder carcinoma

In silico analysis of gene expression data from cancer tissues in the Oncomine and TCGA databases, showed remarkable up-regulation of NUSAP1 gene expression compared to normal bladder tissues. Bladder cancer-derived cell lines were subjected to RNA interference-mediated NUSAP1 knockdown, which reduced the rate of proliferation, cell invasion and migration, and induced cell cycle arrest in mitosis. These effects were related to the inhibition of the epithelial-to-mesenchymal transition via pathways that involved TGF-beta signaling. Interestingly, NUSAP1 down-regulation also potentiated cell death induction by Gemcitabine in baldder carcinoma cell lines.

Colorectal cancer

Immunohistochemical analysis and qRT-PCR assays showed that NUSAP1 protein and mRNA levels are upregulated in colon tumours compared to adjacent non-cancerous tissues (Han et al., 2018; Liu et al., 2018). These data are consistent with those obtained from colorectal cancer (CRC) cell lines (Caco2, LS174T, SW480, and LoVo) by qRT-PCR and WB experiments, suggesting possible roles of NUSAP1 in colorectal cancer progression. Indeed, NUSAP1 abundance is significantly associated with histopathological grading, depth of invasion, lymph node metastasis and TNM stage of CRC, and correlates with poor survival: indeed, an analysis of the overall survival (OS) of CRC patients showed a lower OS rate for patients with high NUSAP1 expression compared to patients with low expression, 5 year after surgery (Liu et al., 2018). High expression of NUSAP1 may therefore be predictive of poor prognosis in CRC.

The role of NUSAP1 in CRC was investigated by post-transcriptional silencing: NUSAP1 knockdown was found to inhibit cell proliferation, migration, invasion, as well as the epithelial-to-mesenchymal transition (EMT), while promoting apoptosis compared to non-interfered cells. Expression of the DNA methyl-transferase (DNMT1) gene, encoding the major enzyme required for both de novo and maintenance cytosine methylation in 5'-CG-3' dinucleotides in genomic DNA, was also inhibited in NUSAP1-silenced cells; given the master role of DNMT1 in establishing the epigenetic landscape of the cells, that finding might account for the global effects observed in NUSAP1-silenced cells; how NUSAP1 might influence DNMT1 expression remains however still unclear (Han et al., 2018). These results suggest that NUSAP1 may be a good candidate target for CRC treatment.

Hepatocellular carcinoma

NUSAP1 has been implicated in liver cancer in a microarray profiling study of microRNAs in mouse models of hepatocellular carcinoma (HCC) and human tumour tissues: the microRNA miRNA 193a-5p was originally found to be downregulated in these samples compared with non-tumour liver tissues. In addition, reduced expression of MIR193A (miRNA 193a-5p) was also found in advanced HCC human samples (stage T3), and low

levels of expression correlate with shorter survival of HCC patients. NUSAP1 was identified in a search for miRNA 193a-5p target genes upregulated in HCC samples. Remarkably, downregulation of NUSAP1 mimics the functional effects of miRNA 193a-5p overexpression in HCC cells. These results identify NUSAP1 as a regulatory target gene among those that the 'master' miRNA 193a-5p implicates in HCC progression.

Oral Squamous Cell Carcinoma

The NUSAP1 gene is upregulated in oral squamous carcinoma cells (OSSC) compared with normal oral keratinocytes. Immunohistochemistry depicts increased levels of NUSAP1 protein in primary OSSC tissues, especially high in advanced compared with early tumour stages. NUSAP1 knockdown causes a significant decrease of proliferation rates in OSSC-derived cells and arrests the cell cycle with a G2/M genomic content. These data suggest that the high levels of NUSAP1 observed in OSSC stimulates these cells to overproliferate by stimulating mitosis, hence accelerating tumour progression. Indeed, NUSAP1 knockdown in combination with Paclitaxel (PTX), an anti-microtubule drug that arrests the mitotic division, enhances PTX-induced apoptosis in OSSC-derived cells. Based on these data, low levels of NUSAP1 might potentiate the efficacy of PTX treatment and might be regarded as an option to improve OSCC therapy.

Gliomas

Data deposited in Oncomine and TGCA databases reveal that NUSAP1 is overexpressed in astrocytoma cell lines and tissues compared with normal astrocytes and brain tissues, and overexpression correlates with poor survival. Ectopic expression or knockdown of NUSAP1 promoted or inhibited, respectively, the proliferative and invasive abilities of astrocytoma cells compared with controls. Furthermore, intracranial xenografts of astrocytoma cells engineered to express NUSAP1 were highly invasive compared with the parental cells. NUSAP1 might therefore represent a potential prognostic biomarker in astrocytoma. In turn, NUSAP1 upregulation in astrocytoma cells causes the nuclear translocation of the Zinc finger protein GLI1, also known as glioma-associated oncogene. Although the molecular mechanism through which NUSAP1 influences the nuclear translocation of GLI1 remains unknown, the data indicate that this results in the activation of GLI1 target genes of the Hedgehog pathway, ultimately enhancing tumour cell invasiveness. NUSAP1 can thus be regarded as a promising target for Astrocytoma therapy.
Glioma samples of various grades also show upregulation of both NUSAP1 transcript and NUSAP1 protein compared to normal tissues (Zhu et al., 2018; Qian et al., 2018). NUSAP1 protein abundance increases with the degree of malignancy and pathological grade of glioma, and patients with high NUSAP1 expression exhibited significantly reduced OS, compared to those with low NUSAP1. Furthermore, in vitro experiments showed that loss of NUSAP1 impairs the proliferation capacity of glioma cells, inducing cell cycle arrest with a G2/M genomic content (Zhu et al., 2018; Qian et al., 2018) and apoptosis. NUSAP1 is also involved in glioma cells migration (Zhu et al., 2018). Qian et al. also demonstrated that, in a xenograft mouse model of GBM (IV degree of glioma), the tumour volume was smaller in NUSAP1-silenced compared with control animals, indicating a role of NUSAP1 in glioma progression in vivo (Qian et al., 2018). On these grounds, high levels of NUSAP1 might be viewed as a new prognostic factor in human Glioblastoma Multiforme (GBM).

Pancreatic adenocarcinoma

Pancreatic adenocarcinoma is an aggressive tumour, often resistant to chemotherapy. Kokkinakis et al. identified two prominent factors inducing resistance to DNA alkylating agents: high levels of O6-methylguanine-DNA methyltransferase (MGMT), and widespread mutations of p53 which impair the induction of cell cycle arrest in the DNA damage response (DDR) (Kokkinakis et al., 2003). An approach to induce cell cycle arrest while, at the same time, eliminating MGMT activity, is represented by methionine deprivation: an analysis of cDNA oligoarray under this condition indicated a pleiotropic response of pancreatic adenocarcinoma cell lines, with down-regulation of NUSAP1 as well as other mitotic genes, causing cell cycle arrest. The simultaneous methionine stress and administration of O6-benzylguanine (MGMT inactivators) increase the sensitivity of pancreatic tumour cell lines to Temozolomide, favouring cancer cell death (Kokkinakis et al., 2005).

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