# Multifaceted role of Tensins in cancer

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## Abstract

Tensins are structural adaptor proteins localized at focal adhesions. Tensins can act as mechanosensors and participate in the transduction of biochemical signals from the extracellular matrix to the cytoskeleton, acting as an interface able to alter cell behavior in responses to changes in their surrounding environment.
This review aims to provide a concise summary of the main functions of the four known tensins in cell and cancer biology, their homology and recently unveiled signaling mechanisms. We focus specifically on how tensin 4 (TNS4/Cten) may contribute to cancer both as an oncogene supporting metastasis and as tumour suppressor in different types of tissue. A better understanding of the cancer mechanistics involving tensins may provide the rationale for development of specific therapeutic strategies.

## Introduction

Tensins were first identified in the 1980s as F-actin (filamentous actin) capping proteins localised to focal adhesions. Later, tensins were considered not only as structural proteins, but also as adaptor proteins linking F-actin to both the cytoskeleton and the extracellular matrix (ECM) (Wilkins & Lin, 1986; Wilkins, Risinger & Lin, 1986; Bockholt & Burridge, 1993; Chuang, Lin & Lin, 1995). The microenvironment can influence cell motility through the mechanical properties of the extracellular matrix (such as density or stiffness) and through growth factors embedded in the extracellular matrix. As adaptor proteins, tensins act as mechanossensors and they can participate in the transduction of biochemical signals from the ECM to the cytoskeleton, hereby altering the behaviour of cells in response to changes in the

extracellular matrix. This review aims to provide insight on the role of different tensins in the development of cancer and, in particular, the recently unveiled mechanisms through which tensins, specifically tensin 4 (Cten) contribute to cancer both as oncogenes (supporting metastasis) and as tumour suppressors.

## Survey methodology

In order to conduct our literature search we have used Pubmed, Google Scholar and Scopus as search engines. The terms to search for relevant published articles included: Tensin 1, Tensin 2, Tensin 3, Tensin 4, Tensins, Cten, Beta-catenin, EGFR. Doctoral thesis available in accessible repositories were also considered for our literature review.

## Homology and evolutionary biology of tensins

All tensins have in common the presence of the SH2 (Src homology 2) and PTB (phosphotyrosine binding) domains. TNS2 has an additional C1 domain (protein kinase C conserved region) at the N-terminus (Hafizi et al., 2010) and TNS4 lacks the actin-binding domain (ABD) at its N-terminus which is present in the other tensins and which is duplicated in TNS1 (Figure 1A) (Lo, 2014). Whilst there is marked evolutionary sequence conservation between all four tensins at the SH2 and PTB domains, homology at the N-terminus is limited to tensins 1, 2 and 3 since TNS4 lacks the majority of this region (which contains the actin-binding domain). TNS4 is only detected in mammals and may be a mammalian-specific gene. The other Tensins are also expressed in mammals and TNS1 expression was further detected in nematodes, zebrafish and hydrozoans (Lo et al., 1997; Chen et al., 2002, 2013a; Cui, Liao & Lo, 2004a). Alignment of coding sequence shows that Tensins 1 and 3 are more closely related to each other than to TNS2 whilst TNS4 is the most distant member (Figure 1B). Individual alignments of the separate domains (SH2, PTB) reflect the same phylogenetic relationship between tensins, suggesting these regions evolved at the same pace as the N-terminus region.

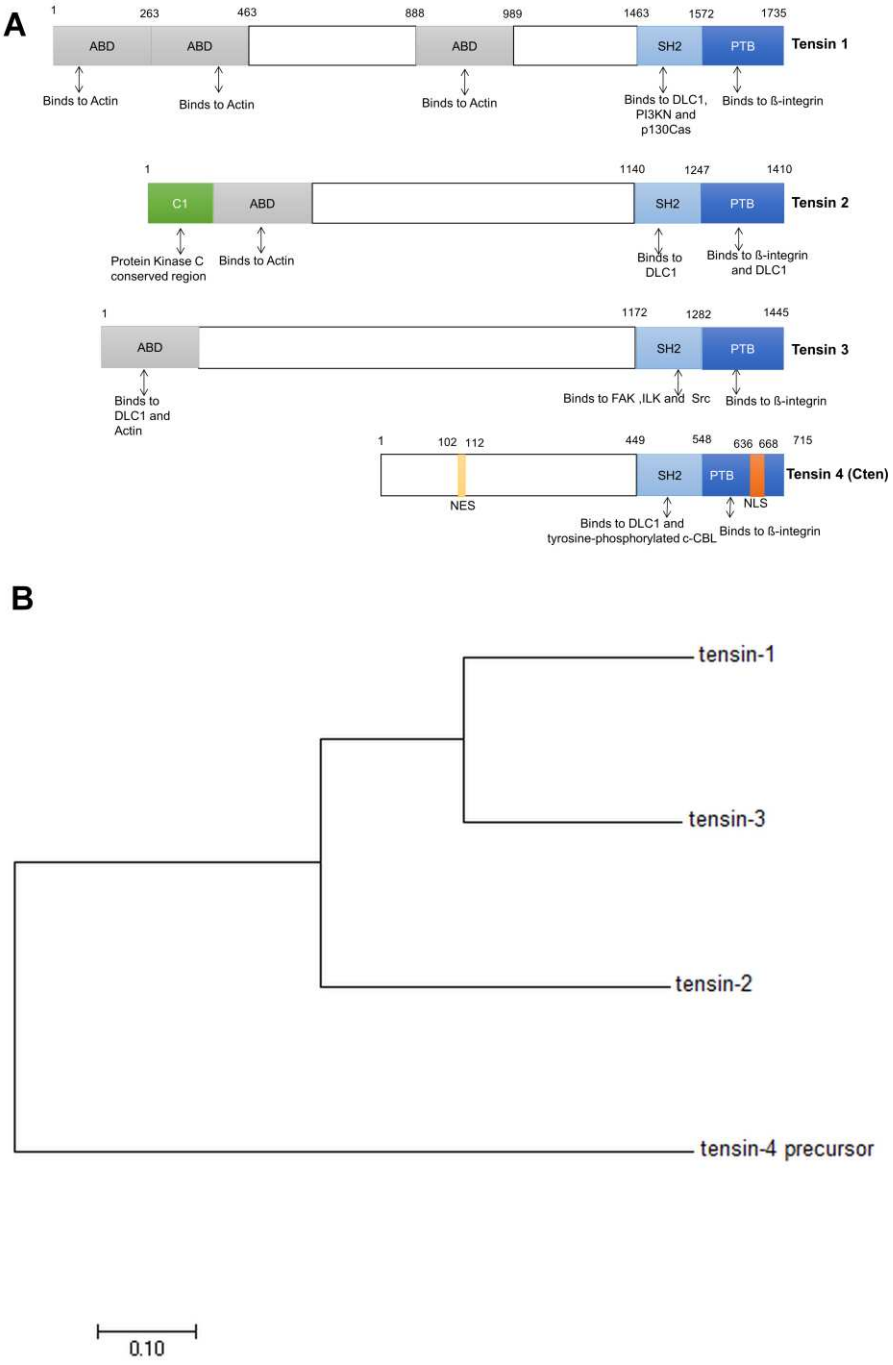


*Figure 1 - (A) Structure of members of the Tensin family. Tensin-1, Tensin-2 and Tensin-3 contain an ABD (actin binding domain), SH2 (Src homology 2) domain and PTB (phosphotyrosine binding domain). Tensin-2 has a conserved protein kinase C region 1 that is not present in other Tensins. TNS4 has a SH2 domain and PTB domain but lacks the ABD. Among all Tensins, Nuclear localization signal (NLS) and Nuclear export signal (NES) was identified in TNS4. (B) Molecular Phylogenetic analysis of tensins by Maximum Likelihood method.*

Tensins 1 – 3 are also close relatives of the tumour suppressor gene Phosphate tensin homolog (PTEN) and they share homology in the N-terminal region C2 domains (responsible for membrane targeting) and protein tyrosine phosphatase (PTP) domains(Haynie, 2014). Other proteins sharing homology with the first N-terminal 150 aa of the tensins 1-3 are the cyclin-G associated kinase (GAK) and Auxilin (Haynie, 2014), although these cannot be classified as members of the tensin family. Although sharing domains with kinases and phosphatases, none of the tensins have been reported to have any enzymatic activity. Our own data have shown that they can be the targets of kinase activity but their biological activity is related to their role as structural proteins (Akhlaq, 2016). To underline the potential impact of structural homology, there is a range of PTEN oncogenic mutations that mimic the structure of tensins and auxilins such as mutations occurring at PTEN P-loops. These are permissive for its activity in vitro and do not affect the enzyme catalytic activity (Rodríguez-Escudero et al., 2011).

The homology of TNS4 across mammalian species has been investigated and a phylogenetic tree of the relationship between TNS4 in domestic mammals shows conservation across species Figure 2. Similarly, the core promoter region of TNS4 was identified as a conserved 327 bp fragment, although alternative TNS4 promoters may exist (Chen et al., 2013b). Within this previously identified region, we have conducted a bioinformatics search and found other putative binding sites for TCF4/LEF1 and C-myc which have yet to be proven experimentally (Raposo et al., 2019 Submitted). TNS4 is expressed at high levels in the prostate and placenta in humans and mice, but low levels of TNS4 can be detected in the lung, brain, skeletal muscle and kidney (Chen et al., 2013a).

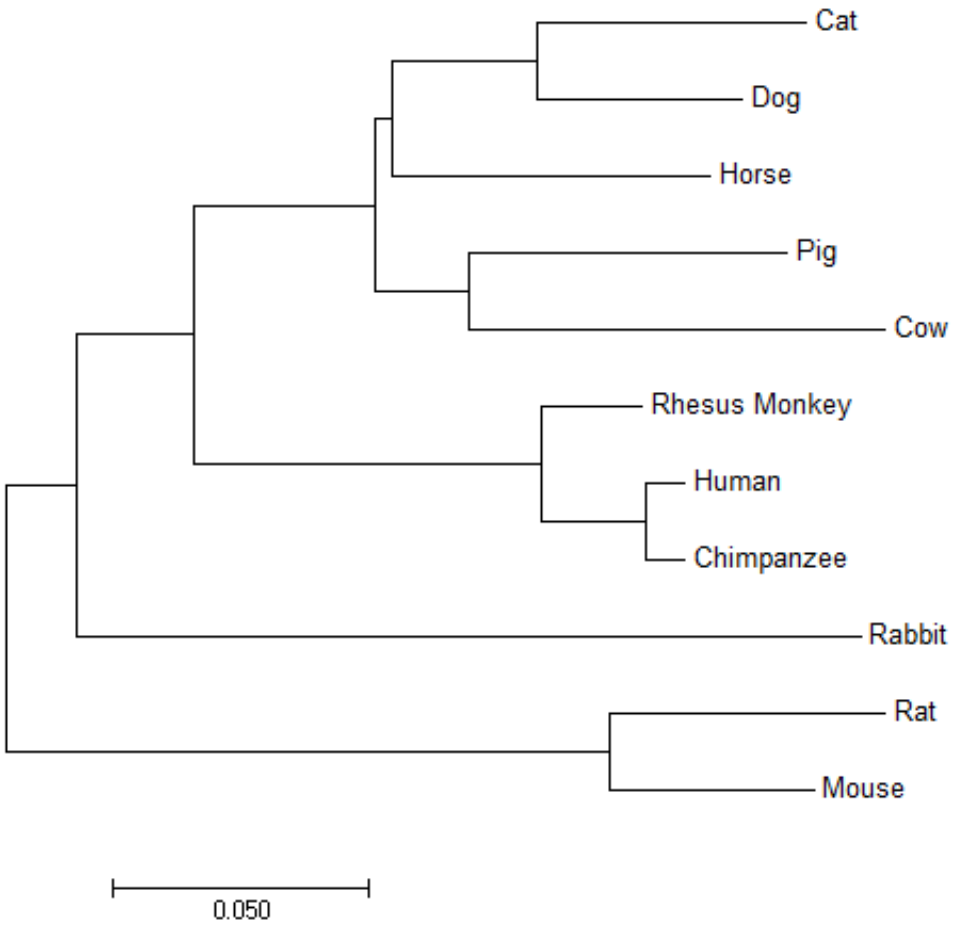


*Figure 2 - Molecular Phylogenetic analysis of tensin 4 in domestic mammals by Maximum Likelihood method.*

The subcellular localization of a protein is important for its biological function (Butler & Overall, 2009). Like other tensin members, TNS4 mainly localizes to focal adhesion complex (Lo, 2014), where it recruits and stabilizes several proteins and enhances cellular functions (Table 1). However, TNS4 has been shown to present in the nucleus (Liao et al., 2009a; Albasri et al., 2011a) suggesting that it may possess a nuclear localization signal (NLS). Hong et al. have recently demonstrated that TNS4 contains a nuclear localization signal (NLS), a nuclear export signal (NES) and two focal binding sites. Deletion of the NLS abolished TNS4 nuclear localization. Moreover, transfecting a TNS4 construct lacking the NES expression led to accumulation of TNS4 in the nucleus and induced HeLa cell proliferation (Hong et al., 2019b). We ourselves have also searched for an NLS and we have discovered a putative NLS protein motif common to tensins 1, 3 and 4 (KLFFRRHYP), but absent in TNS2 (Figure 3). The score of the NLS region in TNS2 representing its likelihood to translocate into the nucleus was also lower (5.4) than for the other tensins (between 6-8) with the highest scoring NLS being that of tensin4,

indicating it is the most likely protein to have nuclear translocation. Even though nuclear translocation of TNS1 and 3 and its effects has not been reported yet, it is very probable these tensins, as in the case of TNS4, signal to the nucleus and shuttle between the nucleus and the cytoplasm.

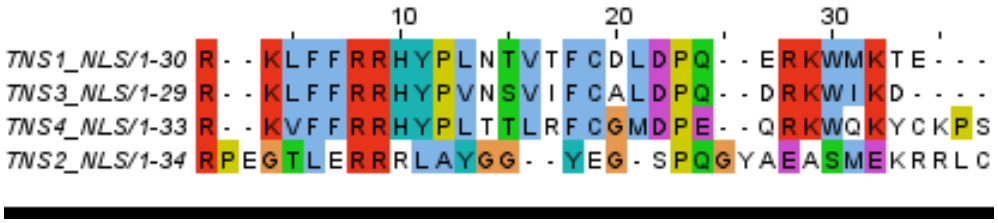


*Figure 3 - Alignment of NLS regions in the four tensins and highlighted conserved amino acid residues.*

Although the tensins are mainly structural proteins without any enzymatic domains, they play a significant role in cell signalling as part of their role in regulating cell adhesion and cellular interaction with the extracellular matrix. Interactions between the four different tensins and epidermal growth factor receptor signalling have been reported. The most explored of these is TNS4 and recently it has been shown that TNS4 is involved in the transduction of extra-celluar signals for a wide variety of growth factors. These data place TNS4 at the heart of a nexus of signalling pathways, the paradigm of which would be EGFR/KRAS/MAPK signalling (Albasri et al., 2011a; Al-Ghamdi et al., 2013; Thorpe et al., 2015; Asiri et al., 2018b, 2019). In this pathway, TNS4 has been shown to mediate its effect through the reduction of EGFR degradation (Hong et al., 2013) by interacting with the phosphotyrosine residues of EGFR through its SH2 domain (Figure 4). This ability to bind to phosphotyrosines in EGFR is also present in both of the SH2 and PTB domains of TNS2, but not those of TNS1 and 3. However, Tensins 1 – 3 are able to interact with phosphotyrosine residues of ErbB2, whereas TNS4 is not (Jones et al., 2006). The complexity of the relationships between the tensins is shown in untransformed breast cells where activation of ErbB2 (a member of the EGFR family) results in a displacement of TNS3 by TNS4 at focal adhesions (called the “tensin switch”) (Katz et al., 2007). The mechanistic basis of this switch is not known but, in colorectal cancer, this switch is not seen and our data shows that in fact levels of both TNS3 and TNS4 increase following EGF stimulation (Thorpe et al., 2015) (Figure 5).

| Organ | *In vitro* assays of TNS4 function | TNS4 expression in Clinical samples |
|---|---|---|
| **Normal Prostate** | Cleaved during apoptosis(Lo, Lo & Lo, 2005).<br>Increases cell adhesion(Yang et al., 2016b).<br>Disturbs acinar formation(Wu & Liao, 2018). | TNS4 expression was higher in three out of four cases when compared to tumour adjacent tissue (Lo & Lo, 2002b). |
| **Normal human skin** | Induces proliferation and possibly differentiation (Seo et al., 2017a). | Localized in basal layer keratinocytes. Downregulated in differentiated keratinocytes (Seo et al., 2017a). |
| **Normal mammary gland, Breast** | Induces cell migration (Katz et al., 2007). | TNS4 expression not detected (Albasri et al., 2011a). |
| **Prostate cancer** | No data reported. | Downregulated in three out of four cases when compared to normal adjacent tissue (Lo & Lo, 2002b).<br>TNS4 protein expression correlated inversely with Gleason score(Li et al., 2010b). |
| **Melanoma** | No data reported. | TNS4 expression correlated positively with tumour thickness and associated with poorer prognosis and response to treatment (Sjoestroem et al., 2013b). |
| **Breast cancer** | Overexpression of TNS4 (571-715) induces apoptosis whereas overexpression of full length TNS4 did not (Lo, Lo & Lo, 2005). | High TNS4 expression associated with tumour size, grade, metastasis, upregulation of EGFR and HER, and shorter cancer-specific and metastasis-free survival (Katz et al., 2007). |
| **Colorectal cancer** | Induces the EMT, promotes cell invasion, migration (Albasri et al., 2009, 2011a; Liao et al., 2009b; Asiri et al., 2019) and cancer stemness (Liao et al., 2009b). | Overexpressed in early and late stage (Liao et al., 2009b).<br>High TNS4 expression associated with a poorer survival rate, metastasis, advanced Duke's stage(Albasri et al., 2011a). |
| **HCC** | Induces proliferation and migration (Chan et al., 2015). | Upregulated in 43% of HCC patient samples (Chan et al., 2015).<br>Association between TNS4 expression and patient outcomes was not reported (Chan et al., 2015). |
| **Lung cancer** | Induces cell proliferation (Muharram et al., 2014b), EMT, invasion and migration (Bennett et al., 2015; Lu et al., 2018). | Significantly upregulated in stage II, III and IV compared to adjacent normal tissue (Sasaki et al., 2003a).<br>Upregulated in adenocarcinoma compared to normal lung (Misono et al., 2019).<br>High TNS4 expression associated with poorer disease-free and overall survival (Misono et al., 2019). |
| **Gastric cancer** | Inhibits apoptosis, induces cell proliferation(Muharram et al., 2014b), migration and invasion (Aratani et al., 2017a). | Upregulated in tumour tissues compared to adjacent normal mucosa. High TNS4 expression associated with poorer 5-year overall survival (Sawazaki et al., 2017). |
| **Cervical cancer** | Nuclear TNS4 induces cell proliferation (Hong et al., 2019a). | No data reported yet. |
| **Esophagogastric cancer** | No data reported. | Upregulated in 34% of 104 primary tumour samples. TNS4 expression is associated with lymphatic invasion, lymph node metastasis, and poorer overall and disease-free survival (Aratani et al., 2017a). |
| **Thymoma** | No data reported. | TNS4 mRNA expression was significantly higher in stage IV when compared to stage I (Sasaki et al., 2003c). |
| **Pancreatic cancer** | Induce cell migration (Al-Ghamdi et al., 2011a). | No data reported. |

*Table 1-Summary of the reported functions of TNS4 in vitro and associations between TNS4 expression in clinical samples and patient outcomes in various types of cancer and normal tissue.*

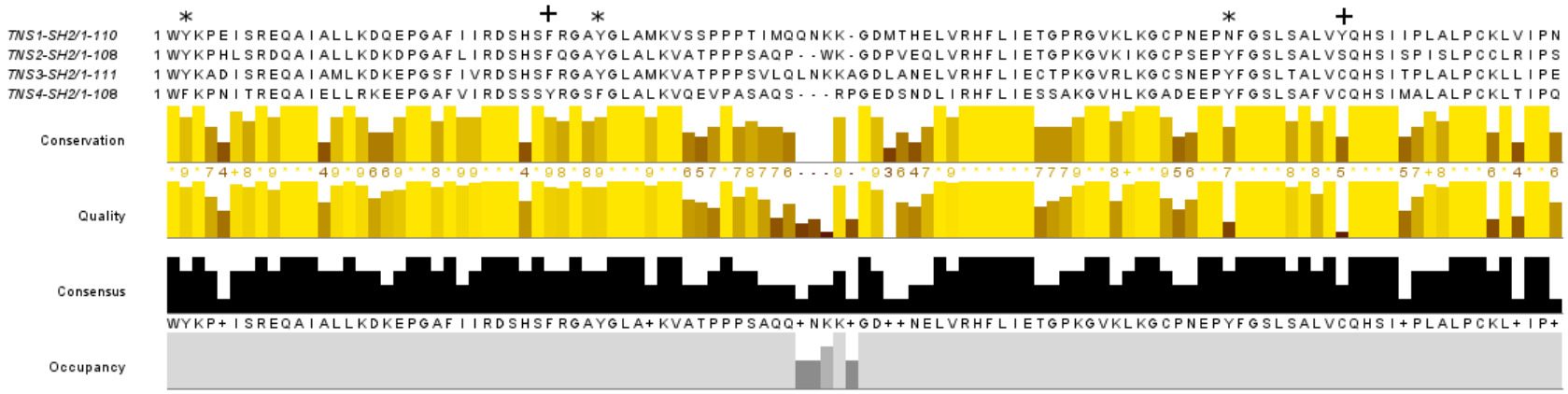


*Figure 4 - Homology for the SH2 domain across the four tensins. High conservation of tyrosine (Y) residues is indicated by * and low conservavtion indicated by +.*

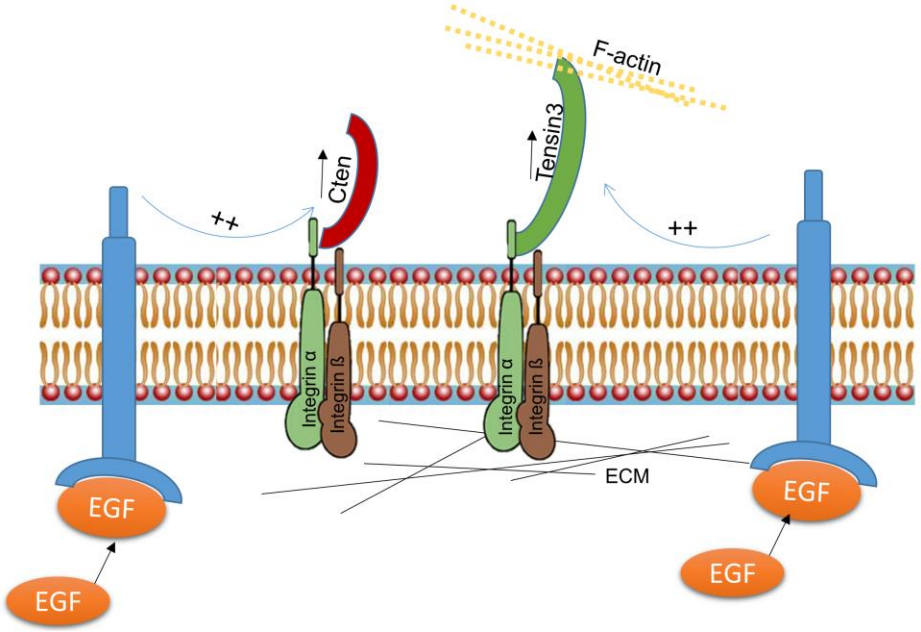


*Figure 5 - TNS3 and TNS4 upregulation following EGF stimulation in colorectal cancer.*

# The role of Tensins in cancer

## Tensin 1

Tensin 1 (TNS1) maps to chromosome 2q35 and encodes a 220kDa tyrosine-phosphorylated protein that contains actin-binding and actin-capping domains. In common with the other tensins, it has an SH2 domain and it has regions with homology to auxilin (a cyclin G associated kinase) and to the PTEN tumour suppressor gene (Chen et al., 2002). Additionally, TNS1 has a C2 tensin-like domain, which together with SH2 and PTB domains, is responsible for binding to DLC1 and enhancing Rho-A activity (Shih et al., 2015). In human primary fibroblasts, TNS1 is required for integrin trafficking to fibrillar adhesions and for fibronectin fibril assembly (Pankov et al., 2000). The phosphotyrosine binding domain (PTB) of TNS1 binds to the NPxY motif in the cytoplasmic tails of integrins ß1A and ß3. During the maturation of focal adhesions, tyrosine phosphorylation of integrin is thought to act as a switch to promote the migration of tensin-integrin complexes into the fibronectin-mediated fibrillar adhesions (McCleverty, Lin & Liddington, 2007).

TNS1 is not required during embryogenesis for the normal development of tissues and organs. In adult mice TNS1 is present in the kidney in the mesangial cells, tubular epthelium and parietal epithelium of the glomerulus. It is necessary for physiological renal function and for the production of extracellular matrix. TNS1-null mice develop multiple large cysts in the renal proximal tubules (Lo et al., 1997; Yamashita et al., 2004).

TNS1 functions partly through its interactions with Deleted in Liver Cancer (DLC) tumour suppressor genes. DLC1 and DLC3 contain RhoGTPase-activating domains which bind to both PTB and SH2 domains of TNS1 simultaneously (Qian et al., 2007). Through this interaction, TNS1 also enhances RhoA activity in a DLC1-dependent manner through a TNS1-DLC1-RhoA signalling axis that regulates endothelial cell proliferation, migration and angiogenesis (Shih et al., 2015) (Figure 6). In addition, the N-terminal domain of TNS1, associates with protein phosphatase-1alpha (PP1alpha) resulting in reduced migration and invasion independently of DLC-1 binding (Eto et al., 2007; Hall et al., 2009). Interestingly, phosphorylation of TNS1 by p38 MAPK regulates the specificity of the SH2 domain for binding to different protein partners such as DLC-1, p130Cas and FAK. Thus, TNS1 may provide an intersection point for many signalling pathways including p38 MAP kinase, tyrosine kinases and RhoGTPases (Hall et al., 2010).

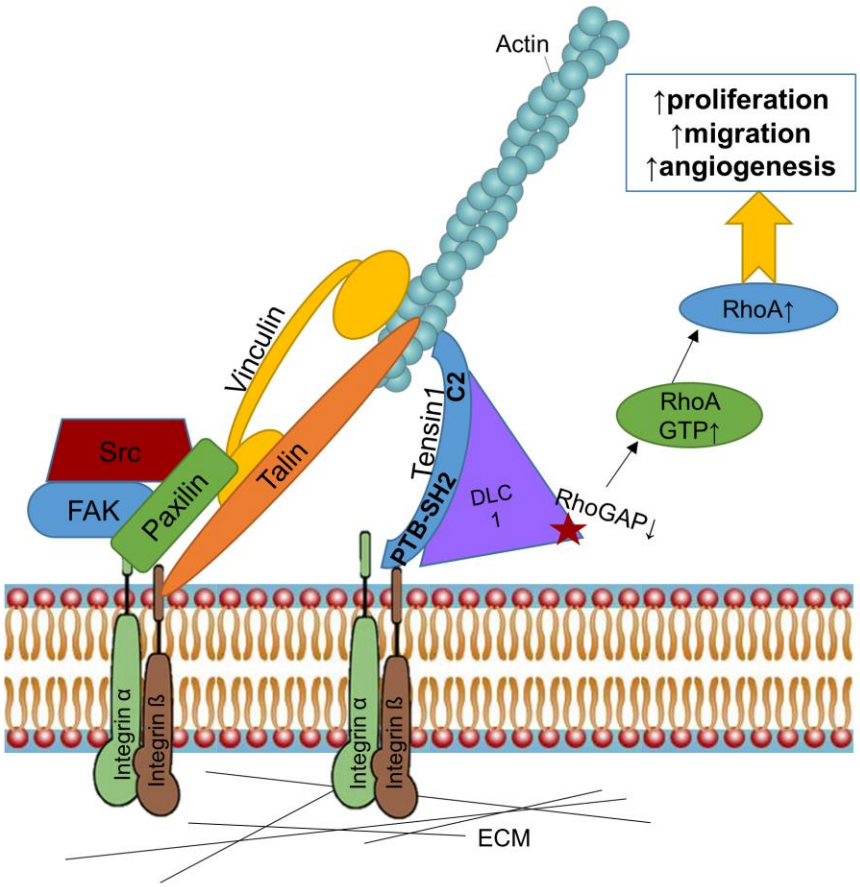


*Figure 6 - TNS1 interaction with DLC1 and effects on RhoA.*

The multiplicity of TNS1activities may be the reason why it has a complex role in cancer development. In renal cancer, expression of all TNS1 seems to be downregulated suggesting a role as tumour-suppressors (Martuszewska et al., 2009). Similarly, in breast cancer cell lines and breast cancer xenografts in mice, TNS1 appears to have a tumour suppressor function albeit through an interesting mechanism. TNS1 was found to harbour two 3'UTR binding sites for the miRNA miR-548j, which functions as a metastasis-promoting miRNA. Binding of miR-548j to these sites appears to regulate promote breast cancer cell invasion and metastasis by down-regulating TNS1 and activating Cdc42 (Zhan et al., 2016).In contrast to renal and breast cancer, TNS1 has been proposed as an oncogene in colorectal cancer (Burghel et al., 2013). Overexpression of TNS1 has been detected in human biopsies of colorectal tumours and reported to be associated with poor prognosis (Zhou et al., 2018).

## Tensin 2

Tensin 2 (TNS2) was identified in the early 2000s as a result of a Blast search for sequences sharing homology with TNS1. TNS2 is mostly conserved at the N-terminal and C-terminal portions, presenting 60% - 70% sequence similarity with TNS1 (Chen et al., 2002). TNS2 has a C1 domain without homology to other tensins, containing a protein C kinase conserved.

Transfection with a tensin2 construct containing this C1 domain or C1 and PTPase domains appear to localize preferentially to the nucleus, suggesting this region has nuclear transport functions (Hafizi et al., 2010).

Expression of TNS2 occurs in a variety of normal human tissues, and is found to be particularly intense in heart, skeletal muscle, kidney and liver (Chen et al., 2002). The SH2 domain of TNS2 has also been reported to influence the phosphorylation of IRS-1 (insulin receptor substrate 1) via binding to phosphatidylinositol-3,4,5-triphosphate, therefore contributing to the regulation of insulin signalling pathways (Kim et al., 2018). (Hong et al., 2016). The SH2 domain of TNS2 also interacts with a short peptide sequence of DLC1 – (CSRLSIYDNVPG) independently of tyrosine phosphorylation (Dai et al., 2011). Binding of TNS2 to DLC1 has been shown to occur via interaction with endogenous caveolin-1, the major component of caveolae, lipid-rich invaginations concentrating regulatory proteins of signal transduction and pathogenesis of various cancers (Williams & Lisanti, 2005). This finding suggested the complex formed between DLC1 and TNS2 could interact with RhoGTPases to alter cytoskeleton organization (Yam et al., 2006b).

By data-mining it has also been reported that in most cancers TNS2 is downregulated, and low TNS2 levels are associated with a poor prognosis in breast and lung cancers, suggesting its role as a tumour suppressor. Similarly to TNS1, the PTB domain of TNS2 interacts with DLC1 to suppress growth of hepatocellular carcinoma cells (Chen et al., 2012). In agreement with that, downregulation of TNS2 causes upregulation of Akt, Mek, and IRS-1 activity, increasing tumorigenicity in A549 and Hela cells (ref). However, The role of TNS2 in cancer is complicated and controversial. For example, overexpression of a variant lacking the C1 region (v3) induced cell migration, proliferation, colony formation and tumour growth in BEL7402 cells, whereas overexpression of a variant with the C1 region (v2) reduced colony formation in HepG2 and BEL7402 liver cancer cell lines(Yam et al., 2006c,a). Since these variant (V2, V3) exerts different functions in BEL7402 cells, it is highly possible that different regions of TNS2 exert different functions

Individual domains of TNS2 may be cleaved by caspases during apoptosis (Kook et al., 2003; Lo, Lo & Lo, 2005) and as a result translocate to different subcellular localizations where they will exert different functional aspects in terms of cell motility and survival (Hafizi et al., 2010). Information on other mechanisms by which TNS2 exerts its tumour suppressor activity are still sparse and require further investigation.

## Tensin 3

Tensin 3 (TNS3) was discovered soon after TNS2 as a new member of the tensin family. TNS3 maps to chromosome 7p12.3 and its expression is ubiquitous in a variety of tissues, but particularly in kidney, lung and placenta (Fagerberg et al., 2014). Similarly to other tensin members, TNS3 localises to focal adhesions where it forms a complex with FAK and p130Cas.

In breast cancer cell lines, stimulation with EGF induces tyrosine phosphorylation of TNS3 requiring both EGFR activation and Src kinase activity, but dissociates the complex formed at the focal adhesion sites, enhancing the interaction with EGFR (Cui, Liao & Lo, 2004b). Therefore, TNS3 is suggested to act as a platform for the disassembly of focal adhesion complexes upon EGF stimulation

TNS3 has been shown to enhance the RhoGAP activity of DLC1 by converting it to an active state (Cao et al., 2012), but under EGF stimulation the dynamics of interaction between DLC1, TNS3, PTEN and PI3K are altered to produce a phosphorylation-mediated molecular switch that controls the spatiotemporal activation of the small GTPases, Rac1 and RhoA. These changes may initiate directional cell migration induced by growth factors (Cao et al., 2015). In contrast, in breast cancer cell lines, activation of DLC (deleted in liver cancer) by TNS3 reduces the anchorage-independent growth of transformed cells (Cao et al., 2012). In a TNS3 knockout mouse model, inactive TNS3 resulted in slow growth and lethality in one third of the homozygous mutants, due to reduced development of the intestine, lung and bone (Chiang et al., 2005).

TNS3 appears to have a role as an oncogene or tumour suppressor depending on the context. In human melanoma, lung and breast cancer cell lines, inhibition of TNS3 was found to significantly reduce cell proliferation, anchorage-independent growth and cell migration (Qian et al., 2009). Moreover, the same group demonstrated that TNS3 expression positively influences the clonogenic activity by activated Src and that the SH2 domain of TNS3 acts as a substrate for Src tyrosine kinase (Qian et al., 2009). In the mammary cancer cell line MDA-MB-468, TNS3 was found to have a role in adhesion, spreading and migration, as shown by TNS3 knockdown which caused disruption of cell-attachment dynamics at focal adhesion sites (Veß et al., 2017). This suggests a role for TNS3 in the initial steps of metastasis when loss of adhesion and the EMT program starts. However, in renal cell carcinoma, high methylation levels at CpG islands were associated with low TNS3 expression levels, but this could be reversed to high TNS3 expression by demethylation of cultured kidney cells, which suggests an epigenetic mechanism for the regulation of TNS3 promoter and its possible role as a tumour suppressor gene in renal cancer (Carter et al., 2013).

In glioblastoma, the RNA-binding protein musashi-1 (MSI-1) was found to bind to the 3'UTR of TNS3 mRNA, to inhibit its translation and increase cell migration and promote changes in cell viscoelasticity, regulated by RhoA-GTP. Moreover, high expression ratio of MSI1 to TNS3 was associated to higher GBM xenograft migration and the authors also demonstrated a mutual exclusion between MSI1 and TNS3 (Chen et al., 2017). This mechanism suggesting a tumour-suppressor activity for TNS3 could however be particular to glioblastoma as conflicting findings were reported in breast cancer (Veß et al., 2017).

## Tensin 4

TNS4 (C-terminal tensin-like) or TNS4 (tensin4) is the most recently described member of the tensin protein family, encoded by chromosome 17q21.2 and localized at focal adhesion sites. It has homology with the other tensins in the SH2 (Src homology 2) and PTB (phosphotyrosine binding) domains at its C-terminus (Lo & Lo, 2002a). In contrast to other tensins, TNS4 lacks an actin binding domain, therefore its role in the dynamics of cell movement and adhesion may be different to the other tensins.

At focal adhesion sites, TNS4, forms complexes with integrinß1 independently of HGF stimulation, TNS4 is able to stabilize and regulates the HGF receptor, Met, by reducing its internalization and lysosomal trafficking (Muharram et al., 2014a). In addition, TNS4 can interact with phosphorylated tyrosine residues through the SH2 domain (Lo, 2007), and thus it can also stabilize and directly interact with phosphorylated Met to increase survival, proliferation, and migration. Our group and others have reported that TNS4 is regulated by numerous cytokines and growth factors. TNS4 is mainly regulated by the MEK-ERK and PI3K-AKT pathways and is upregulated by EGF, FGF2, NGF, PDGF, HGF, TGF-β, IGF-1, IL-6, and IL-13 in a time- and dose-dependent manner (Hung et al., 2014; Muharram et al., 2014b). These growth factors are frequently and widely upregulated in cancer. The ability of FGF2, TFG-β1 and EGF to induce migration is mediated by TNS4 (Hong et al., 2013; Hung et al., 2014; Asiri et al., 2018a). This indicates that TNS4 might be a promising potential therapeutic target for treating cancers associated with upregulation of these growth factors.

Although positive regulation of TNS4 has been extensively reported, as described above, two studies have reported negative regulation of TNS4. TNS4 was identified as a target of miR150-3p in a genome-wide gene expression analysis of lung adenocarcinoma. MiR150 exhibits tumour suppressor activity in a variety of cancers (Koshizuka et al., 2017; Okato et al., 2017; Osako et al., 2017). Overexpression of miR150-3p significantly reduced TNS4 mRNA and protein expression (Sasaki et al., 2003a). Moreover, silencing of the well-characterized tumour suppressor gene P53 resulted in upregulation of TNS4 in a variety of lung cancer cell lines, suggesting that P53 represses TNS4 expression (Barta et al., 2019).

Activation of the Wnt pathway is central to the initiation and progression of CRC . Deregulation of the Wnt signalling pathway and accumulation of Beta-catenin in the nucleus is often the result of adenomatous poliposi coli (APC) mutations disturbing the beta-catenin destruction complex (Novellasdemunt, Antas & Li, 2015) which is present in about 80% of sporadic colorectal tumours (Fearnhead, Wilding & Bodmer, 2002). Our recent data on ApcMin/+ mice adenomas shows TNS4 up-regulation, which we then demonstrate by overexpression or inhibition of Beta-catenin, to be downstream of Wnt signalling (Raposo et al., 2019 submitted). Expression of TNS4 in clinical samples is associated with advanced clinical stage. However, TNS4 mRNA is also overexpressed in all CRC samples tested regardless of the stage, suggesting TNS4 may also

play a role in tumour initiation and progression (Liao et al., 2009a; Albasri et al., 2011a). Interestingly, TNS4 physically interacts with Beta-catenin in CRC cell lines. (Liao et al., 2009b). We have identified an N-terminal region comprising 100 a.a. that, when deleted, abolishes the co-immunoprecipitation with Beta-catenin. However, we have not detected any biological function resulting of that interaction in HCT116. To determine if this interaction plays a role in tumour initiation, we would have to test it in normal colorectal epithelial cells (Alfahed, 2019 in preparation). A simulation of the 3D protein structure of TNS4 and its interaction domains with Beta-catenin shows a long tail interacting with a grove in the armadillo structure of Beta-catenin (Figure 7).

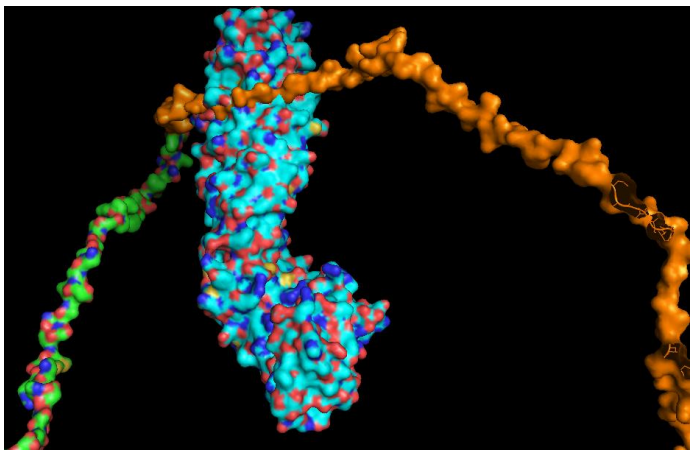

*Figure 7 - Predicted interaction between the N-terminal part of TNS4 and a grove in the armadillo structure of Beta-catenin.*

The involvement of TNS4 in Beta-catenin signalling is apparent as TNS4 seems to regulate expression of Snail and signal through TGF-ß regulating EMT in colorectal cancer cell lines (Thorpe et al., 2017; Asiri et al., 2019), as does Beta-catenin in the nucleus by controlling the transcription factor TCF1 to increase migration and reduce adhesion (Vincan & Barker, 2008). Nuclear localization of TNS4 has been previously identified in metastasis of colorectal cancer (Albasri et al., 2011a). Interestingly, upregulation of TNS4 was also identified in the molecular signature defining the rounded cell phenotype within a HCT-8 population which resembles the initiation of metastasis (Tang et al., 2014). Similarly, TNS4 has also been featured in a molecular signature of cancer stem cells derived from human induced pluripotent stem cells conditioned by the supernatant of cancer cell lines (Seno et al., 2016), which despite the artificiality of the conditions created, points to a role for TNS4 in the events of neoplastic transformation.

A physical interaction between TNS4 and EGFR has not been yet demonstrated but, as previously mentioned, TNS4 is upregulated by EGF stimulation (Figure 5) and stabilizes EGFR by reducing its ligand-induced degradation (Katz et al., 2007; Hong et al., 2013; Chan et al., 2015). Interestingly, EGFR was also identified in a search conducted on Biogrid as the only molecule appearing in the interactomes of both TNS4 and Beta-catenin (Figure 5). It is therefore

a possibility that TNS4 and Beta-catenin interact not only directly with each other but also as mutual partners of EGFR (Figure 8).

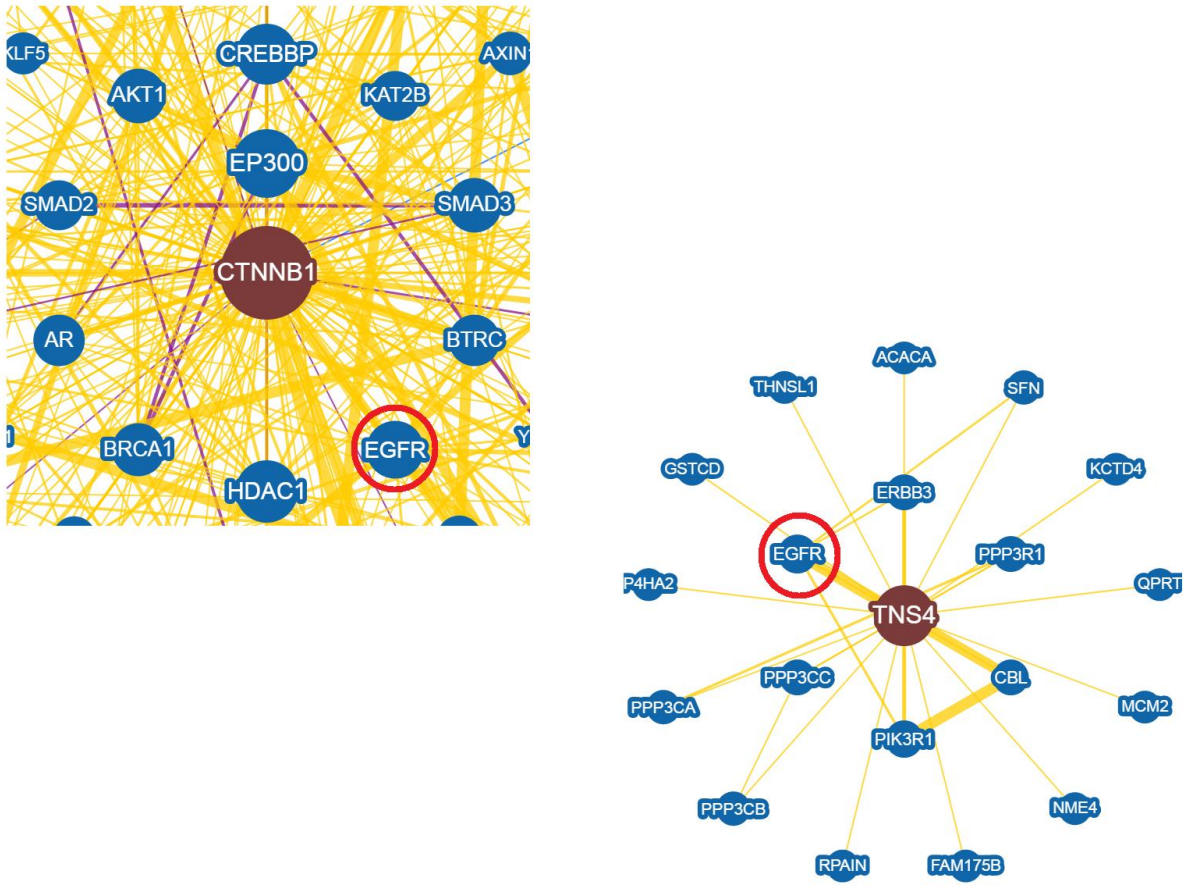


*Figure 8 - Network of Beta-catenin and TNS4 interactors (thebiogrid.org). EGFR (circled in red) is a common interactor for both proteins.*

In the normal prostatic epithelium, induction of apoptosis results in cleavage of TNS4 at residue 570 by caspase-3. Transfection of the cleaved form (571-715 of TNS4) reduces the growth rate, but not the proliferation of breast cancer cells, suggesting that this cleaved TNS4 fragment may play a role in induction of apoptosis, possibly by diminished activity of the PTB domain (Lo, Lo & Lo, 2005). In agreement with that finding, overexpression of TNS4 increased paclitaxel sensitivity whereas downregulation of TNS4 decreased paclitaxel sensitivity in prostate cancer cells (Li et al., 2010a). Cleavage of tensins and cytoskeleton disruption has also been observed during yessotoxin-induced apoptosis (Korsnes et al., 2007), suggesting this is a common mechanism across the tensin family.

In keeping with the other tensins, the role of TNS4 in cancer appears to be organ-dependent (Table 1). It may function as a tumour suppressor gene in prostate cancer. A probably pathogenic non-synonymous mutation T427C has been recently reported in prostate cancer which is thought

to upregulate STAT1 and its nuclear translocation (Chen et al., 2018). Similarly, TNS4 is under the control of ΔNp63 in normal prostate cells and expression of both TNS4 and ΔNp63 decreases sequentially from normal prostate to primary prostatic tumours to metastatic lesions (Yang et al., 2016c). Interestingly, mRNA expression levels of all tensins are downregulated in renal cancer, compared to normal kidney tissue, implying its unusual role as a tumour suppressor gene in renal cancer (Martuszewska et al., 2009).

In contrast to the prostate, TNS4 has been suggested as a putative oncogene due to its overexpression in a vast range of cancer types: breast, colorectal, hepatocellular carcinoma, melanoma, oesophagogastric adenocarcinoma, gastric, pancreatic, thymoma and lung cancer (Sasaki et al., 2003b; Sakashita et al., 2008; Albasri et al., 2009, 2011a; Al-Ghamdi et al., 2011b; Sjoestroem et al., 2013a; Chen et al., 2014; Aratani et al., 2017b; Sawazaki et al., 2017).

In CRC cell lines, TNS4 has been shown to induce EMT by repressing E-cadherin and to significantly increase migration and invasion (Albasri et al., 2009). TNS4 also appears to regulate ILK (integrin-linked kinase) expression and TNS4-induced cell motility depends in part on ILK (Albasri et al., 2011a). The ILK-FAK axis is downstream of the Twist-Integrin beta 1 pathways that mediate EMT in human mammary epithelium and breast cancer cells (Yang et al., 2016a), so it is possible that TNS4 functions as one of the intermediate effectors in EMT, regulating the integrin response to extrinsic stimulants of EMT. Similarly, a study in non-small cell lung cancer cell lines has shown that TNS4 overexpression determines TGF-ß increased activity and conversely TNS4 knockdown reverses the effect of TGF-ß stimulation in EMT markers, migration and invasion assays (Lu et al., 2018). Our research group has recently reported a role for TNS4 in mediating EMT induced by TGF-ß stimulation in colorectal cancer cell lines. In the absence of TNS4, the effects of TGF-ß stimulation on inducing motility and invasion were abrogated, but not its role on inducing proliferation (Asiri et al., 2019). TNS4 has been shown to induce cell migration and invasion in CRC cell lines in vitro (Liao et al., 2009a; Albasri et al., 2011a). To confirm this effect in vivo, TNS4 stably transfected cells and empty vector control-transfected cells were injected into nude mice. While the number of spleen and liver tumours were similar between groups, the cells stably expressing TNS4 formed larger tumours compared to control cells. Moreover, the control group had longer overall survival compared to the mice injected with TNS4 stably expressing cells (Albasri et al., 2011a).

The tensin switch described by Katz et al in a mammary cell line with TNS4 upregulation and Tensin3 downregulation, could not be verified in CRC cell lines (Thorpe et al., 2015). Indeed, activation of EGFR pathway through amplification of EGFR is unlikely in CRC. Yet, gain-of-function mutations in KRAS/BRAF (EGFR downstream targets) are detected in 60% of CRC tumours (Seth et al., 2009; Fadhil et al., 2010). However, TNS4 mRNA and protein are also regulated by KRAS in CRC and pancreatic cancer cell lines. The ability of KRAS to induce TNS4 expression was mediated by BRAF. Overexpression of TNS4 restored the reduction in cancer cell migration caused by knockdown of KRAS. These data suggest that TNS4 mediates

the cellular migration induced by KRAS mutations (Al-Ghamdi et al., 2011). TNS4 expression was strongly induced by EGF in a variety of lung cancer cell lines; however, this process could be prevented by inhibition of STAT3 (Bennett et al., 2015). An in-vivo study using a knock-in Stat3C mouse model demonstrated increased migration, invasion and metastatic ability in tumour-derived mammary cancer cell lines mediated by STAT3-dependent over-expression of TNS4 (Pensa et al., 2012). Collectively, the evidence indicates that TNS4 is a common downstream target of the EGFR pathway. EGFR can activate TNS4 via the STAT3 and RAS-MAPK pathways. Moreover, activated KRAS—another downstream target of the EGFR pathway—can upregulate TNS4 in CRC cell lines. These data suggest TNS4 is a promising therapeutic target for tumours with high EGFR pathway activity.

In lung cancer cell lines (Muharram et al., 2014a). TNS4 was demonstrated to regulate integrinß1 and Met levels, and also to be required for Met associated cell survival and proliferation in vitro and in vivo (Muharram et al., 2014a). In keratinocytes, TNS4 was also found to regulate cell proliferation via regulation of integrinß4 -FAK-ERK pathway (Seo et al., 2017b). However in colorectal cancer cell lines, a positive effect of TNS4 overexpression over cell proliferation was not observed (Albasri et al., 2009).

Several tensins, including TNS4, interact with cytoplasmic signalling molecules containing phosphorylated tyrosine residues through the SH2 domain (Lo, 2007), such as Met (hepatocyte growth factor receptor). TNS4 seems to stabilize and directly interact with phosphorylated Met via its SH2-domain to increase survival, proliferation, and migration. Concomitantly, TNS4 and Met immunoexpression correlates in colon and ovarian carcinomas (Muharram et al., 2014a).

## Conclusions and future perspectives

With this literature review we have provided a summary of the main functions of tensins 1-4 in cell and cancer biology, their homology and signalling mechanisms which may provide the rationale for development of specific therapeutic strategies. In the case of TNS4, we have highlighted its interactions with Beta-catenin and EGFR and discussed the relevance of its regulation by Wnt signalling. Whilst the oncogenic function of TNS4 in colorectal cancer is yet warranting in vivo demonstration, its involvement at the core of several signalling pathways hints at a determinant role in signal transduction for induction of cell motility, invasion and EMT. In Colorectal cancer in particular, TNS4 might be a valuable addition to sensitize cancer cells to EGFR inhibition therapies in KRAS mutant patients (Kim et al., 2019), and therefore development and research of new drugs targeting TNS4 is encouraged. Due to the growing body of evidence suggesting TNS4 overexpression has prognostic value in a varied range of malignancies (Sasaki et al., 2003a; Albasri et al., 2011b; Sjoestroem et al., 2013a; Lo, 2014; Chen et al., 2014; Aratani et al., 2017b), it is expected that in a foreseeable future the first pre-clinical trials targeting TNS4 will emerge.

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## References

Akhlaq M. 2016. Investigation of Cten signalling and regulation in colorectal cancer. PhD Thesis. University of Nottingham.

Al-Ghamdi S, Albasri A, Cachat J, Ibrahem S, Muhammad BA, Jackson D, Nateri AS, Kindle KB, Ilyas M. 2011a. Cten is targeted by Kras signalling to regulate cell motility in the colon and pancreas. PLoS One 6:e20919. DOI: 10.1371/journal.pone.0020919.

Al-Ghamdi S, Albasri A, Cachat J, Ibrahem S, Muhammad BA, Jackson D, Nateri AS, Kindle KB, Ilyas M. 2011b. Cten Is Targeted by Kras Signalling to Regulate Cell Motility in the Colon and Pancreas. PLoS ONE 6:e20919. DOI: 10.1371/journal.pone.0020919.

Al-Ghamdi S, Cachat J, Albasri A, Ahmed M, Jackson D, Zaitoun A, Guppy N, Otto WR, Alison MR, Kindle KB, Ilyas M. 2013. C-Terminal Tensin-Like Gene Functions as an Oncogene and Promotes Cell Motility in Pancreatic Cancer. Pancreas 42:135–140. DOI: 10.1097/MPA.0b013e3182557ceb.

Albasri A, Al-Ghamdi S, Fadhil W, Aleskandarany M, Liao Y-C, Jackson D, Lobo DN, Lo SH, Kumari R, Durrant L, Watson S, Kindle KB, Ilyas M. 2011a. Cten signals through integrin-linked kinase (ILK) and may promote metastasis in colorectal cancer. Oncogene 30:2997–3002. DOI: 10.1038/onc.2011.26.

Albasri A, Aleskandarany M, Benhasouna A, Powe DG, Ellis IO, Ilyas M, Green AR. 2011b. CTEN (C-terminal tensin-like), a novel oncogene overexpressed in invasive breast carcinoma of poor prognosis. Breast cancer research and treatment 126:47–54. DOI: 10.1007/s10549-010-0890-3.

Albasri A, Seth R, Jackson D, Benhasouna A, Crook S, Nateri AS, Chapman R, Ilyas M. 2009. C-terminal Tensin-like (CTEN) is an oncogene which alters cell motility possibly through repression of E-cadherin in colorectal cancer. Journal of Pathology. DOI: 10.1002/path.2508.

Aratani K, Komatsu S, Ichikawa D, Ohashi T, Miyamae M, Okajima W, Imamura T, Kiuchi J, Nishibeppu K, Kosuga T, Konishi H, Shiozaki A, Fujiwara H, Okamoto K, Tsuda H, Otsuji E. 2017a. Overexpression of CTEN relates to tumor malignant potential and poor outcomes of adenocarcinoma of the esophagogastric junction. Oncotarget 8:84112–84122. DOI: 10.18632/oncotarget.21109.

Aratani K, Komatsu S, Ichikawa D, Ohashi T, Miyamae M, Okajima W, Imamura T, Kiuchi J, Nishibeppu K, Kosuga T, Konishi H, Shiozaki A, Fujiwara H, Okamoto K, Tsuda H, Otsuji E. 2017b. Overexpression of CTEN relates to tumor malignant potential and poor outcomes of adenocarcinoma of the esophagogastric junction. Oncotarget 8:84112–84122. DOI: 10.18632/oncotarget.21109.

Asiri A, Raposo TP, Alfahed A, Ilyas M. 2018a. TGFbeta1-induced cell motility but not cell proliferation is mediated through Cten in colorectal cancer. Int J Exp Pathol 99:323–330. DOI: 10.1111/iep.12300.

Asiri A, Raposo TP, Alfahed A, Ilyas M. 2018b. TGFβ1-induced cell motility but not cell proliferation is mediated through Cten in colorectal cancer. International Journal of Experimental Pathology 99:323–330. DOI: 10.1111/iep.12300.

Asiri A, Raposo TP, Alfahed A, Ilyas M. 2019. TGFβ1-induced cell motility but not cell proliferation is mediated through Cten in colorectal cancer. International Journal of Experimental Pathology. DOI: 10.1111/iep.12300.

Barta JA, Pauley K, Kossenkov A V, McMahon SB. 2019. The lung-enriched p53 mutants V157F and R158L/P regulate a gain of function transcriptome in lung cancer. Carcinogenesis. DOI: 10.1093/carcin/bgz087.

Bennett DT, Reece TB, Foley LS, Sjoberg A, Meng X, Fullerton DA, Weyant MJ. 2015. C-terminal tensin-like protein mediates invasion of human lung cancer cells and is regulated by signal transducer and activator of transcription 3. J Thorac Cardiovasc Surg 149:369–375. DOI: 10.1016/j.jtcvs.2014.08.087.

Bockholt SM, Burridge K. 1993. Cell spreading on extracellular matrix proteins induces tyrosine phosphorylation of tensin. The Journal of biological chemistry 268:14565–7.

Burghel GJ, Lin W-Y, Whitehouse H, Brock I, Hammond D, Bury J, Stephenson Y, George R, Cox A. 2013. Identification of Candidate Driver Genes in Common Focal Chromosomal Aberrations of Microsatellite Stable Colorectal Cancer. PLoS ONE 8:e83859. DOI: 10.1371/journal.pone.0083859.

Butler GS, Overall CM. 2009. Proteomic identification of multitasking proteins in unexpected locations complicates drug targeting. Nat Rev Drug Discov 8:935–948. DOI: 10.1038/nrd2945.

Cao X, Kaneko T, Li JS, Liu A-D, Voss C, Li SSC. 2015. A phosphorylation switch controls the spatiotemporal activation of Rho GTPases in directional cell migration. Nature Communications 6:7721. DOI: 10.1038/ncomms8721.

599 Cao X, Voss C, Zhao B, Kaneko T, Li SS-C. 2012. Differential regulation of the activity of
600 deleted in liver cancer 1 (DLC1) by tensins controls cell migration and transformation.
601 Proceedings of the National Academy of Sciences 109:1455–1460. DOI:
602 10.1073/pnas.1114368109.

603 Carter JA, Górecki DC, Mein CA, Ljungberg B, Hafizi S. 2013. CpG dinucleotide-specific
604 hypermethylation of the TNS3 gene promoter in human renal cell carcinoma. Epigenetics 8:739–
605 747. DOI: 10.4161/epi.25075.

606 Chan L-K, Chiu Y-T, Sze KM-F, Ng IO-L. 2015. Tensin4 is up-regulated by EGF-induced
607 ERK1/2 activity and promotes cell proliferation and migration in hepatocellular carcinoma.
608 Oncotarget 6:20964–76. DOI: 10.18632/oncotarget.4122.

609 Chen H, Duncan IC, Bozorgchami H, Lo SH. 2002. Tensin1 and a previously undocumented
610 family member, tensin2, positively regulate cell migration. Proceedings of the National Academy
611 of Sciences 99:733–738. DOI: 10.1073/pnas.022518699.

612 Chen NT, Kuwabara Y, Conley C, Liao YC, Hong SY, Chen M, Shih YP, Chen HW, Hsieh F,
613 Lo SH. 2013a. Phylogenetic analysis, expression patterns, and transcriptional regulation of
614 human CTEN gene. Gene 520:90–97. DOI: 10.1016/j.gene.2013.02.041.

615 Chen N-T, Kuwabara Y, Conley C, Liao Y-C, Hong S-Y, Chen M, Shih Y-P, Chen H-W, Hsieh
616 F, Lo SH. 2013b. Phylogenetic analysis, expression patterns, and transcriptional regulation of
617 human CTEN gene. Gene 520:90–97. DOI: 10.1016/j.gene.2013.02.041.

618 Chen H-Y, Lin L-T, Wang M-L, Laurent B, Hsu C-H, Pan C-M, Jiang W-R, Chen P-Y, Ma H-I,
619 Chen Y-W, Huang P-I, Chiou A, Chiou S-H. 2017. Musashi-1 Enhances Glioblastoma Cell
620 Migration and Cytoskeletal Dynamics through Translational Inhibition of Tensin3. Scientific
621 Reports 7:8710. DOI: 10.1038/s41598-017-09504-7.

622 Chen L, Liu C, Ko FCF, Xu N, Ng IO, Yam JWP, Zhu G. 2012. Solution Structure of the
623 Phosphotyrosine Binding (PTB) Domain of Human Tensin2 Protein in Complex with Deleted in
624 Liver Cancer 1 (DLC1) Peptide Reveals a Novel Peptide Binding Mode. Journal of Biological
625 Chemistry 287:26104–26114. DOI: 10.1074/jbc.M112.360206.

626 Chen X-B, Wang F, Pi Y, Zhang Y, Liu P, Lu S. 2018. A novel TNS4 mutant in prostate cancer
627 cells and its mechanism | CHEN | TUMOR. Tumor 38. DOI: 10.3781/j.issn.1000-
628 7431.2018.11.625.

629 Chen J, Zhang Y, Deng G, Ma J, Wu X, Qu Y, Zeng S. 2014. [Correlation between the
630 expression of C-terminal tensin-like protein and the prognosis of hepatocellular carcinoma].
631 Zhong nan da xue xue bao. Yi xue ban = Journal of Central South University. Medical sciences
632 39:1233–9. DOI: 10.11817/j.issn.1672-7347.2014.12.003.

633 Chiang M-K, Liao Y-C, Kuwabara Y, Lo SH. 2005. Inactivation of tensin3 in mice results in
634 growth retardation and postnatal lethality. Developmental Biology 279:368–377. DOI:
635 10.1016/j.ydbio.2004.12.027.

636 Chuang JZ, Lin DC, Lin S. 1995. Molecular cloning, expression, and mapping of the high
637 affinity actin-capping domain of chicken cardiac tensin. The Journal of cell biology 128:1095–
638 109.

639 Cui Y, Liao YC, Lo SH. 2004a. Epidermal growth factor modulates tyrosine phosphorylation of
640 a novel tensin family member, tensin3. Mol Cancer Res 2:225–232.

641 Cui Y, Liao Y-C, Lo SH. 2004b. Epidermal growth factor modulates tyrosine phosphorylation of
642 a novel tensin family member, tensin3. Molecular cancer research : MCR 2:225–32.

643 Dai K, Liao S, Zhang J, Zhang X, Tu X. 2011. Solution Structure of Tensin2 SH2 Domain and
644 Its Phosphotyrosine-Independent Interaction with DLC-1. PLoS ONE 6:e21965. DOI:
645 10.1371/journal.pone.0021965.

646 Eto M, Kirkbride J, Elliott E, Lo SH, Brautigan DL. 2007. Association of the Tensin N-terminal
647 Protein-tyrosine Phosphatase Domain with the α Isoform of Protein Phosphatase-1 in Focal
648 Adhesions. Journal of Biological Chemistry 282:17806–17815. DOI: 10.1074/jbc.M700944200.

649 Fadhil W, Ibrahem S, Seth R, Ilyas M. 2010. Quick-multiplex-consensus (QMC)-PCR followed
650 by high-resolution melting: a simple and robust method for mutation detection in formalin-fixed
651 paraffin-embedded tissue. J Clin Pathol 63:134–140. DOI: 10.1136/jcp.2009.070508.

652 Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M,
653 Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjöstedt E, Lundberg E, Szigyarto CA-K,
654 Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C,
655 Danielsson F, Mardinoglu A, Sivertsson Å, von Feilitzen K, Forsberg M, Zwahlen M, Olsson I,
656 Navani S, Huss M, Nielsen J, Ponten F, Uhlén M. 2014. Analysis of the Human Tissue-specific
657 Expression by Genome-wide Integration of Transcriptomics and Antibody-based Proteomics.
658 Molecular & Cellular Proteomics 13:397–406. DOI: 10.1074/mcp.M113.035600.

659 Fearnhead NS, Wilding JL, Bodmer WF. 2002. Genetics of colorectal cancer: hereditary aspects
660 and overview of colorectal tumorigenesis. British medical bulletin 64:27–43.

661 Hafizi S, Sernstad E, Swinny JD, Gomez MF, Dahlbäck B. 2010. Individual domains of Tensin2
662 exhibit distinct subcellular localisations and migratory effects. The International Journal of
663 Biochemistry & Cell Biology 42:52–61. DOI: 10.1016/j.biocel.2009.09.005.

Hall EH, Balsbaugh JL, Rose KL, Shabanowitz J, Hunt DF, Brautigan DL. 2010. Comprehensive Analysis of Phosphorylation Sites in Tensin1 Reveals Regulation by p38MAPK. Molecular & Cellular Proteomics 9:2853–2863. DOI: 10.1074/mcp.M110.003665.

Hall EH, Daugherty AE, Choi CK, Horwitz AF, Brautigan DL. 2009. Tensin1 Requires Protein Phosphatase-1α in Addition to RhoGAP DLC-1 to Control Cell Polarization, Migration, and Invasion. Journal of Biological Chemistry 284:34713–34722. DOI: 10.1074/jbc.M109.059592.

Haynie DT. 2014. Molecular physiology of the tensin brotherhood of integrin adaptor proteins. Proteins: Structure, Function, and Bioinformatics 82:1113–1127. DOI: 10.1002/prot.24560.

Hong S-Y, Shih Y-P, Li T, Carraway KL, Lo SH. 2013. CTEN Prolongs Signaling by EGFR through Reducing Its Ligand-Induced Degradation. Cancer Research 73:5266–5276. DOI: 10.1158/0008-5472.CAN-12-4441.

Hong SY, Shih YP, Lo A, Lo SH. 2019a. Identification of subcellular targeting sequences of Cten reveals its role in cell proliferation. Biochim Biophys Acta Mol Cell Res 1866:450–458. DOI: 10.1016/j.bbamcr.2018.10.008.

Hong S-Y, Shih Y-P, Lo A, Lo SH. 2019b. Identification of subcellular targeting sequences of Cten reveals its role in cell proliferation. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1866:450–458. DOI: 10.1016/j.bbamcr.2018.10.008.

Hong S-Y, Shih Y-P, Sun P, Hsieh W-J, Lin W-C, Lo SH. 2016. Down-regulation of tensin2 enhances tumorigenicity and is associated with a variety of cancers. Oncotarget 7:38143–38153. DOI: 10.18632/oncotarget.9411.

Hung SY, Shih YP, Chen M, Lo SH. 2014. Up-regulated cten by FGF2 contributes to FGF2-mediated cell migration. Mol Carcinog 53:787–792. DOI: 10.1002/mc.22034.

Jones RB, Gordus A, Krall JA, MacBeath G. 2006. A quantitative protein interaction network for the ErbB receptors using protein microarrays. Nature 439:168–174. DOI: 10.1038/nature04177.

Katz M, Amit I, Citri A, Shay T, Carvalho S, Lavi S, Milanezi F, Lyass L, Amariglio N, Jacob-Hirsch J, Ben-Chetrit N, Tarcic G, Lindzen M, Avraham R, Liao YC, Trusk P, Lyass A, Rechavi G, Spector NL, Lo SH, Schmitt F, Bacus SS, Yarden Y. 2007. A reciprocal tensin-3-cten switch mediates EGF-driven mammary cell migration. Nature Cell Biology. DOI: 10.1038/ncb1622.

Kim, Kim, Kang, Kim, Won, Cho. 2019. Whole Transcriptome Analysis Identifies TNS4 as a Key Effector of Cetuximab and a Regulator of the Oncogenic Activity of KRAS Mutant Colorectal Cancer Cell Lines. Cells 8:878. DOI: 10.3390/cells8080878.

695 Kim E, Kim D-H, Singaram I, Jeong H, Koh A, Lee J, Cho W, Ryu SH. 2018. Cellular
696 phosphatase activity of C1-Ten/Tensin2 is controlled by Phosphatidylinositol-3,4,5-triphosphate
697 binding through the C1-Ten/Tensin2 SH2 domain. Cellular Signalling 51:130–138. DOI:
698 10.1016/j.cellsig.2018.07.009.

699 Kook S, Kim DH, Shim SR, Kim W, Chun JS, Song WK. 2003. Caspase-dependent cleavage of
700 tensin induces disruption of actin cytoskeleton during apoptosis. Biochemical and biophysical
701 research communications 303:37–45.

702 Korsnes MS, Hetland DL, Espenes A, Aune T. 2007. Cleavage of tensin during cytoskeleton
703 disruption in YTX-induced apoptosis. Toxicology in Vitro 21:9–15. DOI:
704 10.1016/j.tiv.2006.07.012.

705 Koshizuka K, Nohata N, Hanazawa T, Kikkawa N, Arai T, Okato A, Fukumoto I, Katada K,
706 Okamoto Y, Seki N. 2017. Deep sequencing-based microRNA expression signatures in head and
707 neck squamous cell carcinoma: Dual strands of premiR- 150 as antitumor miRNAs. Oncotarget
708 8:30288–30304. DOI: 10.18632/oncotarget.16327.

709 Li Y, Mizokami A, Izumi K, Narimoto K, Shima T, Zhang J, Dai J, Keller ET, Namiki M.
710 2010a. CTEN/tensin 4 expression induces sensitivity to paclitaxel in prostate cancer. Prostate
711 70:48–60. DOI: 10.1002/pros.21037.

712 Li Y, Mizokami A, Izumi K, Narimoto K, Shima T, Zhang J, Dai J, Keller ET, Namiki M.
713 2010b. CTEN/tensin 4 expression induces sensitivity to paclitaxel in prostate cancer. The
714 Prostate 70:48–60. DOI: 10.1002/pros.21037.

715 Liao Y-C, Chen N-T, Shih Y-P, Dong Y, Lo SH. 2009a. Up-regulation of C-terminal tensin-like
716 molecule promotes the tumorigenicity of colon cancer through beta-catenin. Cancer research
717 69:4563–6. DOI: 10.1158/0008-5472.CAN-09-0117.

718 Liao YC, Chen NT, Shih YP, Dong Y, Su H Lo. 2009b. Up-regulation of C-terminal tensin-like
719 molecule promotes the tumorigenicity of colon cancer through β-catenin. Cancer Research. DOI:
720 10.1158/0008-5472.CAN-09-0117.

721 Lo SH. 2007. Reverse interactomics: from peptides to proteins and to functions. ACS chemical
722 biology 2:93–5. DOI: 10.1021/cb700013q.

723 Lo SH. 2014. C-terminal tensin-like (CTEN): A promising biomarker and target for cancer.
724 International Journal of Biochemistry and Cell Biology. DOI: 10.1016/j.biocel.2014.04.003.

725 Lo SH, Lo T Bin. 2002a. Cten, a COOH-terminal tensin-like protein with prostate restricted
726 expression, is down-regulated in prostate cancer. Cancer Research.

727 Lo SH, Lo TB. 2002b. Cten, a COOH-terminal tensin-like protein with prostate restricted
728 expression, is down-regulated in prostate cancer. Cancer Res 62:4217–4221.

729 Lo S-S, Lo SH, Lo SH. 2005. Cleavage of cten by caspase-3 during apoptosis. Oncogene
730 24:4311–4. DOI: 10.1038/sj.onc.1208571.

731 Lo SH, Yu QC, Degenstein L, Chen LB, Fuchs E. 1997. Progressive kidney degeneration in
732 mice lacking tensin. The Journal of cell biology 136:1349–61.

733 Lu X, Gao J, Zhang Y, Zhao T, Cai H, Zhang T. 2018. CTEN induces epithelial-mesenchymal
734 transition (EMT) and metastasis in non small cell lung cancer cells. PLOS ONE 13:e0198823.
735 DOI: 10.1371/journal.pone.0198823.

736 Martuszewska D, Ljungberg B, Johansson M, Landberg G, Oslakovic C, Dahlbäck B, Hafizi S.
737 2009. Tensin3 Is a Negative Regulator of Cell Migration and All Four Tensin Family Members
738 Are Downregulated in Human Kidney Cancer. PLoS ONE 4:e4350. DOI:
739 10.1371/journal.pone.0004350.

740 McCleverty CJ, Lin DC, Liddington RC. 2007. Structure of the PTB domain of tensin1 and a
741 model for its recruitment to fibrillar adhesions. Protein Science 16:1223–1229. DOI:
742 10.1110/ps.072798707.

743 Misono S, Seki N, Mizuno K, Yamada Y, Uchida A, Sanada H, Moriya S, Kikkawa N,
744 Kumamoto T, Suetsugu T, Inoue H. 2019. Molecular Pathogenesis of Gene Regulation by the
745 miR-150 Duplex: miR-150-3p Regulates TNS4 in Lung Adenocarcinoma. Cancers (Basel) 11.
746 DOI: 10.3390/cancers11050601.

747 Muharram G, Sahgal P, Korpela T, DeFranceschi N, Kaukonen R, Clark K, Tulasne D, Carpén
748 O, Ivaska J. 2014a. Tensin-4-dependent MET stabilization is essential for survival and
749 proliferation in carcinoma cells. Developmental Cell. DOI: 10.1016/j.devcel.2014.03.024.

750 Muharram G, Sahgal P, Korpela T, De Franceschi N, Kaukonen R, Clark K, Tulasne D, Carpen
751 O, Ivaska J. 2014b. Tensin-4-Dependent MET Stabilization Is Essential for Survival and
752 Proliferation in Carcinoma Cells. Dev Cell 29:629–630. DOI: 10.1016/j.devcel.2014.05.018.

753 Novellasdemunt L, Antas P, Li VSW. 2015. Targeting Wnt signaling in colorectal cancer. A
754 Review in the Theme: Cell Signaling: Proteins, Pathways and Mechanisms. American journal of
755 physiology. Cell physiology 309:C511-21. DOI: 10.1152/ajpcell.00117.2015.

756 Okato A, Arai T, Yamada Y, Sugawara S, Koshizuka K, Fujimura L, Kurozumi A, Kato M,
757 Kojima S, Naya Y, Ichikawa T, Seki N. 2017. Dual Strands of Pre-miR-149 Inhibit Cancer Cell
758 Migration and Invasion through Targeting FOXM1 in Renal Cell Carcinoma. International
759 journal of molecular sciences 18. DOI: 10.3390/ijms18091969.

760 Osako Y, Seki N, Koshizuka K, Okato A, Idichi T, Arai T, Omoto I, Sasaki K, Uchikado Y, Kita
761 Y, Kurahara H, Maemura K, Natsugoe S. 2017. Regulation of SPOCK1 by dual strands of pre-
762 miR-150 inhibit cancer cell migration and invasion in esophageal squamous cell carcinoma.
763 Journal of human genetics 62:935–944. DOI: 10.1038/jhg.2017.69.

764 Pankov R, Cukierman E, Katz BZ, Matsumoto K, Lin DC, Lin S, Hahn C, Yamada KM. 2000.
765 Integrin dynamics and matrix assembly: tensin-dependent translocation of alpha(5)beta(1)
766 integrins promotes early fibronectin fibrillogenesis. The Journal of cell biology 148:1075–90.

767 Pensa S, Demaria M, Avalle L, Barbieri I, Camporeale A, Poli V. 2012. From tissue invasion to
768 glucose metabolism: the many aspects of signal transducer and activator of transcription 3 pro-
769 oncogenic activities. Hormone Molecular Biology and Clinical Investigation 10:217–25. DOI:
770 10.1515/hmbci-2012-0006.

771 Qian X, Li G, Asmussen HK, Asnaghi L, Vass WC, Braverman R, Yamada KM, Popescu NC,
772 Papageorge AG, Lowy DR. 2007. Oncogenic inhibition by a deleted in liver cancer gene requires
773 cooperation between tensin binding and Rho-specific GTPase-activating protein activities.
774 Proceedings of the National Academy of Sciences 104:9012–9017. DOI:
775 10.1073/pnas.0703033104.

776 Qian X, Li G, Vass WC, Papageorge A, Walker RC, Asnaghi L, Steinbach PJ, Tosato G, Hunter
777 K, Lowy DR. 2009. The Tensin-3 Protein, Including its SH2 Domain, Is Phosphorylated by Src
778 and Contributes to Tumorigenesis and Metastasis. Cancer Cell 16:246–258. DOI:
779 10.1016/j.ccr.2009.07.031.

780 Rodríguez-Escudero I, Oliver MD, Andrés-Pons A, Molina M, Cid VJ, Pulido R. 2011. A
781 comprehensive functional analysis of PTEN mutations: implications in tumor- and autism-related
782 syndromes. Human Molecular Genetics 20:4132–4142. DOI: 10.1093/hmg/ddr337.

783 Sakashita K, Mimori K, Tanaka F, Kamohara Y, Inoue H, Sawada T, Hirakawa K, Mori M.
784 2008. Prognostic relevance of Tensin4 expression in human gastric cancer. Annals of surgical
785 oncology 15:2606–13. DOI: 10.1245/s10434-008-9989-8.

786 Sasaki H, Moriyama S, Mizuno K, Yukiue H, Konishi A, Yano M, Kaji M, Fukai I, Kiriyama M,
787 Yamakawa Y, Fujii Y. 2003a. Cten mRNA expression was correlated with tumor progression in
788 lung cancers. Lung cancer (Amsterdam, Netherlands) 40:151–5.

789 Sasaki H, Yukiue H, Kobayashi Y, Fukai I, Fujii Y. 2003b. Cten mRNA Expression Is
790 Correlated with Tumor Progression in Thymoma. Tumor Biology 24:271–274. DOI:
791 10.1159/000076141.

792 Sasaki H, Yukiue H, Kobayashi Y, Fukai I, Fujii Y. 2003c. Cten mRNA expression is correlated
793 with tumor progression in thymoma. Tumour Biol 24:271–274. DOI: 10.1159/000076141.

794 Sawazaki S, Oshima T, Sakamaki K, Aoyama T, Sato T, Shiozawa M, Yoshikawa T, Rino Y,
795 Imada T, Masuda M. 2017. Clinical Significance of Tensin 4 Gene Expression in Patients with
796 Gastric Cancer. In Vivo 31:1065–1071. DOI: 10.21873/invivo.11171.

797 Seno A, Kasai T, Ikeda M, Vaidyanath A, Masuda J, Mizutani A, Murakami H, Ishikawa T,
798 Seno M. 2016. Characterization of Gene Expression Patterns among Artificially Developed
799 Cancer Stem Cells Using Spherical Self-Organizing Map. Cancer Informatics 15:CIN.S39839.
800 DOI: 10.4137/CIN.S39839.

801 Seo EY, Jin SP, Kim YK, Lee H, Han S, Lee DH, Chung JH. 2017a. Integrin-beta4-TNS4-Focal
802 Adhesion Kinase Signaling Mediates Keratinocyte Proliferation in Human Skin. J Invest
803 Dermatol 137:763–766. DOI: 10.1016/j.jid.2016.10.039.

804 Seo EY, Jin S-P, Kim YK, Lee H, Han S, Lee DH, Chung JH. 2017b. Integrin-β4–TNS4–Focal
805 Adhesion Kinase Signaling Mediates Keratinocyte Proliferation in Human Skin. Journal of
806 Investigative Dermatology 137:763–766. DOI: 10.1016/j.jid.2016.10.039.

807 Seth R, Crook S, Ibrahem S, Fadhil W, Jackson D, Ilyas M. 2009. Concomitant mutations and
808 splice variants in KRAS and BRAF demonstrate complex perturbation of the Ras/Raf signalling
809 pathway in advanced colorectal cancer. Gut 58:1234–1241. DOI: 10.1136/gut.2008.159137.

810 Shih Y-P, Sun P, Wang A, Lo SH. 2015. Tensin1 positively regulates RhoA activity through its
811 interaction with DLC1. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research
812 1853:3258–3265. DOI: 10.1016/j.bbamcr.2015.09.028.

813 Sjoestroem C, Khosravi S, Zhang G, Martinka M, Li G. 2013a. C-terminal tensin-like protein is
814 a novel prognostic marker for primary melanoma patients. PloS one 8:e80492. DOI:
815 10.1371/journal.pone.0080492.

816 Sjoestroem C, Khosravi S, Zhang G, Martinka M, Li G. 2013b. C-terminal tensin-like protein is
817 a novel prognostic marker for primary melanoma patients. PLoS One 8:e80492. DOI:
818 10.1371/journal.pone.0080492.

819 Tang X, Kuhlenschmidt TB, Li Q, Ali S, Lezmi S, Chen H, Pires-Alves M, Laegreid WW, Saif
820 TA, Kuhlenschmidt MS. 2014. A mechanically-induced colon cancer cell population shows
821 increased metastatic potential. Molecular Cancer 13:131. DOI: 10.1186/1476-4598-13-131.

822 Thorpe H, Akhlaq M, Jackson D, Ghamdi S Al, Storr S, Martin S, Ilyas M. 2015. Multiple
823 pathways regulate Cten in colorectal cancer without a Tensin switch. International Journal of
824 Experimental Pathology. DOI: 10.1111/iep.12154.

825 Thorpe H, Asiri A, Akhlaq M, Ilyas M. 2017. Cten promotes epithelial-mesenchymal transition
826 through the post-transcriptional stabilization of Snail. Molecular Carcinogenesis 56:2601–2609.
827 DOI: 10.1002/mc.22704.

828 Veß A, Blache U, Leitner L, Kurz ARM, Ehrenpfordt A, Sixt M, Posern G. 2017. A dual
829 phenotype of MDA-MB-468 cancer cells reveals mutual regulation of tensin3 and adhesion
830 plasticity. Journal of Cell Science 130:2172–2184. DOI: 10.1242/jcs.200899.

831 Vincan E, Barker N. 2008. The upstream components of the Wnt signalling pathway in the
832 dynamic EMT and MET associated with colorectal cancer progression. Clinical & Experimental
833 Metastasis 25:657–663. DOI: 10.1007/s10585-008-9156-4.

834 Wilkins JA, Lin S. 1986. A re-examination of the interaction of vinculin with actin. The Journal
835 of cell biology 102:1085–92.

836 Wilkins JA, Risinger MA, Lin S. 1986. Studies on proteins that co-purify with smooth muscle
837 vinculin: identification of immunologically related species in focal adhesions of nonmuscle and
838 Z-lines of muscle cells. The Journal of cell biology 103:1483–94.

839 Williams TM, Lisanti MP. 2005. Caveolin-1 in oncogenic transformation, cancer, and
840 metastasis. American Journal of Physiology-Cell Physiology 288:C494–C506. DOI:
841 10.1152/ajpcell.00458.2004.

842 Wu WM, Liao YC. 2018. Downregulation of C-Terminal Tensin-Like Protein (CTEN)
843 Suppresses Prostate Cell Proliferation and Contributes to Acinar Morphogenesis. Int J Mol Sci
844 19. DOI: 10.3390/ijms19103190.

845 Yam JW, Ko FC, Chan CY, Jin DY, Ng IO. 2006a. Interaction of deleted in liver cancer 1 with
846 tensin2 in caveolae and implications in tumor suppression. Cancer Res 66:8367–8372. DOI:
847 10.1158/0008-5472.CAN-05-2850.

848 Yam JWP, Ko FCF, Chan C-Y, Jin D-Y, Ng IO-L. 2006b. Interaction of Deleted in Liver Cancer
849 1 with Tensin2 in Caveolae and Implications in Tumor Suppression. Cancer Research 66:8367–
850 8372. DOI: 10.1158/0008-5472.CAN-05-2850.

851 Yam JW, Ko FC, Chan CY, Yau TO, Tung EK, Leung TH, Jin DY, Ng IO. 2006c. Tensin2
852 variant 3 is associated with aggressive tumor behavior in human hepatocellular carcinoma.
853 Hepatology 44:881–890. DOI: 10.1002/hep.21339.

854 Yamashita M, Horikoshi S, Asanuma K, Takahara H, Shirato I, Tomino Y. 2004. Tensin is
855 potentially involved in extracellular matrix production in mesangial cells. Histochemistry and
856 Cell Biology 121:245–254. DOI: 10.1007/s00418-004-0626-8.

Yang J, Hou Y, Zhou M, Wen S, Zhou J, Xu L, Tang X, Du Y, Hu P, Liu M. 2016a. Twist induces epithelial-mesenchymal transition and cell motility in breast cancer via ITGB1-FAK/ILK signaling axis and its associated downstream network. The International Journal of Biochemistry & Cell Biology 71:62–71. DOI: 10.1016/j.biocel.2015.12.004.

Yang K, Wu WM, Chen YC, Lo SH, Liao YC. 2016b. DeltaNp63alpha Transcriptionally Regulates the Expression of CTEN That Is Associated with Prostate Cell Adhesion. PLoS One 11:e0147542. DOI: 10.1371/journal.pone.0147542.

Yang K, Wu W-M, Chen Y-C, Lo SH, Liao Y-C. 2016c. ΔNp63α Transcriptionally Regulates the Expression of CTEN That Is Associated with Prostate Cell Adhesion. PLOS ONE 11:e0147542. DOI: 10.1371/journal.pone.0147542.

Zhan Y, Liang X, Li L, Wang B, Ding F, Li Y, Wang X, Zhan Q, Liu Z. 2016. MicroRNA-548j functions as a metastasis promoter in human breast cancer by targeting Tensin1. Molecular Oncology 10:838–849. DOI: 10.1016/j.molonc.2016.02.002.

Zhou H, Zhang Y, Wu L, Xie W, Li L, Yuan Y, Chen Y, Lin Y, He X. 2018. Elevated transgelin/TNS1 expression is a potential biomarker in human colorectal cancer. Oncotarget 9:1107–1113. DOI: 10.18632/oncotarget.23275.