

The Tensin-3 Protein, Including its SH2 Domain, Is Phosphorylated by Src and Contributes to Tumorigenesis and Metastasis

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SUMMARY

In cell lines from advanced lung cancer, breast cancer, and melanoma, endogenous tensin-3 contributes to cell migration, anchorage-independent growth, and tumorigenesis. Although SH2 domains have not been reported previously to be phosphorylated, the tensin-3 SH2 domain is a physiologic substrate for Src. Tyrosines in the SH2 domain contribute to the biological activity of tensin-3, and phosphorylation of these tyrosines can regulate ligand binding. In a mouse breast cancer model, tensin-3 tyrosines are phosphorylated in a Src-associated manner in primary tumors, and experimental metastases induced by tumor-derived cell lines depend on endogenous tensin-3. Thus, tensin-3 is implicated as an oncoprotein regulated by Src and possessing an SH2 domain with a previously undescribed mechanism for the regulation of ligand binding.

INTRODUCTION

It is widely recognized that most cancers result from a multistep genetic and epigenetic process that includes the inactivation of tumor suppressor genes that inhibit growth and/or promote apoptosis as well as the activation of oncogenes that promote growth and/or inhibit apoptosis (Vogelstein and Kinzler, 2004). Members of the *DLC* gene family (*DLC1-3*) of tumor suppressors are growth inhibitory and proapoptotic and are inactivated in a variety of tumors (Durkin et al., 2007). DLC proteins are found in focal adhesions and can reduce the activity of Rho GTPases via the Rho GTPase activating protein (Rho-GAP) domain present in each DLC protein. Another antioncogenic activity of DLC is its ability to bind the SH2 and PTB domains of tensin proteins, which are found in focal adhesions (Liao et al., 2007; Qian et al., 2007; Yam et al., 2006a).

Mammals contain four tensin genes: *tensin-1-3* and *cten* (*tensin 2* is also designated *C1-ten*; *cten*, for C-terminal tensin-like protein, a truncated *cten* is also designated tensin-4) (Hafizi et al., 2005; Lo, 2004). The proteins encoded by *tensin-1-3* form a link between the actin cytoskeleton and the intracellular portion of some β -integrins, and are believed to participate in the regulation of cell migration. Actin binding is mediated by sequences in the N terminus of tensin-1-3, whereas integrin binding has been localized to the PTB domain at their C terminus (Figure 1D). The SH2 domain of tensin-1-3 lies just upstream of the PTB domain, with unique sequences comprising the middle of each protein. *Cten*, which is shorter than tensin-1-3, lacks the actin binding sequences, but contains SH2 and PTB domains at its C terminus, analogous to tensin-1-3.

SH2 domains are approximately 100 amino acids in length and serve primarily as docking sites for the binding of signaling

SIGNIFICANCE

The role of focal adhesion (FA) proteins in cancer is poorly understood. Here, tensin-3 is found to be an FA protein that contributes to oncogenesis in human cancer cell lines and lines from a mouse cancer model. In addition, tyrosines in the SH2 domain of tensin-3 contribute to its transforming activity. SH2 domains participate in tyrosine kinase signaling pathways and bind ligands that are usually tyrosine phosphorylated, but we find that the tyrosines in the SH2 domain of tensin-3 have in addition the previously undescribed property of being phosphorylated, by Src, an FA-associated non-receptor tyrosine kinase activated in many cancers. This SH2 phosphorylation can facilitate the binding of SH2 ligands such as p130Cas and FAK.

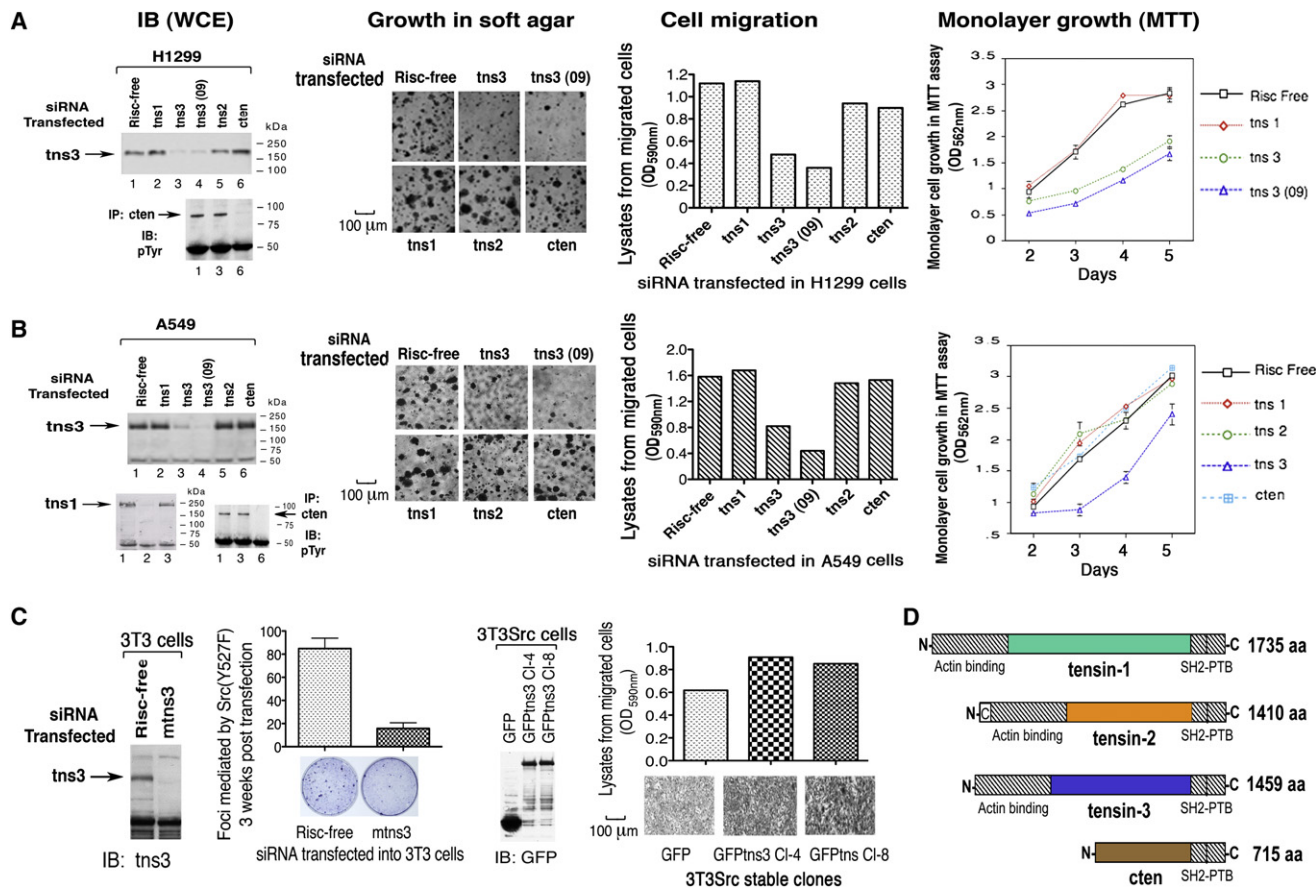


Figure 1. siRNA for Tensin-3, But Not for Other Tensins, Specifically Inhibits Cell Proliferation, Anchorage-Independent Growth, and Cell Migration

(A and B) NSCLC lines 1299 and A549: immunoblots of tensin-3 (tns3) and cten from whole-cell extracts (WCE) resulting from siRNA transfections; bioassays carried out after treatment with the indicated siRNAs for growth in agar, cell migration (scale bars represent 100 μ m), and MTT (shown in graph as mean $\pm$ SD). No detectable tns1 by immunoblot in H1299 cells (data not shown).

(C) Cooperation of tns3 with SrcY527F in cell transformation and migration. Focus formation induced by SrcY527F was reduced as shown by mouse tns3 siRNA transfection in NIH 3T3 cells (left panels, graphed as mean and standard deviation [$\pm$ SD]); cell migration was increased by overexpression of GFP-tns3 in 3T3Src cells, as shown by transwell migration assay (right panels, scale bars represent 100 μ m).

(D) Schematic representation of the four tensin proteins.

molecules involved in tyrosine kinase-dependent pathways (Liu et al., 2006; Machida and Mayer, 2005). Most SH2 domains have several physiologic ligands, whose tyrosine phosphorylation is usually required for efficient binding to their cognate SH2 motifs (Huang et al., 2008). The tensin SH2 domain can form a complex with several tyrosine phosphorylated proteins, including p130Cas and FAK, which are pro-oncogenic factors (Cui et al., 2004; Defilippi et al., 2006; Mitra and Schlaepfer, 2006). However, unphosphorylated DLC can efficiently bind the tensin SH2 domain (Liao et al., 2007), and DLC can compete with other ligands for occupying the SH2 domain of tensin and with β -integrin for binding the PTB domain (Qian et al., 2007).

The ability of the tensin SH2 domain to bind pro-oncogenic ligands raises the possibility that under conditions permissive for such binding, a tensin protein might contribute to the oncogenic phenotype. However, as is true for most focal adhesion proteins (Lindberg et al., 2008), a role for tensin in tumors has not been clearly identified. For example, overexpression of

tensin-1 has been reported to promote cell migration (Chen et al., 2002), a feature of many cancer cells, but tensin-1 may be silenced in human breast and prostate cancer cell lines (Chen et al., 2000). Although full-length tensin-2 can inhibit cell migration and growth, and promote apoptosis (Hafizi et al., 2005; Yam et al., 2006a), overexpression of an alternatively spliced form of tensin-2 can promote cell growth and transformation (Yam et al., 2006b), with both forms often being coregulated in tumor cell lines (Yam et al., 2006a; Yam et al., 2006b). Cten was reported downregulated in most prostate tumor cell lines (Lo and Lo, 2002), and its overexpression in a breast cancer line had no phenotype (Lo and Lo, 2005). Other studies describe cten as contributing to EGF-dependent cell migration in MCF10A cells, a nontransformed human breast cell line, and that it was expressed in breast cancer (Katz et al., 2007).

For this analysis of tensin in cancer, we focused on the role of endogenous tensin protein in cell lines derived from advanced human non-small cell lung cancer (NSCLC), breast cancer, and

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