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Tristetraprolin: Roles in Cancer and Senescence

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Abstract

Cancer and senescence are both complex transformative processes that dramatically alter many features of cell physiology and their interactions with surrounding tissues. Developing the wide range of cellular features characteristic of these conditions requires profound alterations in global gene expression patterns, which can be achieved by suppressing, activating, or uncoupling cellular gene regulatory pathways. Many genes associated with the initiation and development of tumors are regulated at the level of mRNA decay, frequently through the activity of AU-rich mRNA-destabilizing elements (AREs) located in their 3'-untranslated regions. As such, cellular factors that recognize and control the decay of ARE-containing mRNAs can influence tumorigenic or senescent phenotypes mediated by products of these transcripts. In this review, we discuss evidence showing how suppressed expression and/or activity of the ARE-binding protein tristetraprolin (TTP) can contribute to these processes. Next, we outline current findings linking TTP suppression to exacerbation of individual tumorigenic phenotypes, and the roles of specific TTP substrate mRNAs in mediating these effects. Finally, we survey potential mechanisms that cells may employ to suppress TTP expression in cancer, and propose potential diagnostic and therapeutic strategies that may exploit the relationship between TTP expression and tumor progression or senescence.

1. Hallmarks and progression of cancer

1.1 The diverse cellular features of cancer

Decades of research have defined cancer as a disease caused by dynamic changes in the genome. Mutations acquired familially or accumulated over time can activate oncogenes through dominant gain of function and disable tumor suppressor genes by recessive loss of function (Hanahan and Weinberg, 2011). The unstable genome of a tumor cell not only makes cancer unique to each patient, but also provides a mechanism by which a single tumor can be heterogeneous in composition. This pathological heterogeneity in turn complicates strategies for early detection and optimal treatment of many cancers, prompting research efforts to identify biomarkers and cellular mechanisms associated with oncogenic transformation and tumor development that may be exploited for therapeutic intervention.

Although cancers display diverse characteristics among different tissues and individuals, there are several common cellular phenotypes that are acquired during the development and progression of the disease. Six of these “cancer hallmarks” were described in a seminal

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review by Hanahan and Weinberg as: [i] limitless replicative potential, [ii] evasion of apoptosis, [iii] sustained angiogenesis, [iv] self-sufficiency in growth signals, [v] insensitivity to anti-growth signals, and [vi] tissue invasion and metastasis (Hanahan and Weinberg, 2000). More recently, the development of a sustained pro-inflammatory tumor microenvironment has been accepted as a seventh hallmark of cancer (Colotta et al., 2009). Finally, recently identified biological features that drive cancer development were introduced as “emerging hallmarks” in a comprehensively updated review by Hanahan and Weinberg. These were: [i] deregulation of cellular energetics, [ii] genome instability and mutation, and [iii] avoidance of immune detection (Hanahan and Weinberg, 2011). Following from this model, a major focus of current cancer research is to identify specific genetic mechanisms and pathways responsible for the acquisition and manifestation of these tumorigenic phenotypes, which subsequently may provide potential targets for downstream therapeutic development.

1.2 Oncogenes and pathway deregulation

Cancer is caused primarily by somatic mutations of proto-oncogenes, proteins whose mutation or uncontrolled expression will initiate a neoplastic transformation (Croce, 2008). A proto-oncogene can be activated into an oncogene through a myriad of mechanisms including structural alterations, gene fusions, juxtaposition with enhancers, or amplification (Konopka et al., 1985; Tsujimoto et al., 1985). This initial mutation can ultimately lead to deregulation of key growth and apoptosis pathways by directly deregulating a growth pathway or by disrupting genome caretaker pathways, predisposing the cell to further somatic mutations (Konopka et al., 1985). However, oncogenic activation can more widely and radically impact cell physiology through downstream effects on gene expression. Common instances of this phenomenon occur when transcription factors are overexpressed or constitutively activated in cancers, since enhanced activity of these factors can distort the expression of myriad gene targets leading to global alterations in gene regulatory networks. This “transformation amplification process” provides a mechanism by which mutations in a very limited population of genes can dramatically modulate a plethora of cellular phenotypes. For example, the transcription factor MYC is overexpressed in many high-grade pre-malignancies and invasive tumors and is associated with poor patient outcome. MYC directly regulates transcription of many genes that encode proteins involved in promoting cell proliferation, survival, and angiogenesis, while other MYC target genes encode factors that block differentiation and induce genetic instability (Wolfer and Ramaswamy, 2011).

Deregulation of gene regulatory circuits can also result from aberrant activation of cellular signaling pathways, as is often observed when growth factor receptors are mutated. In lung cancer and glioblastoma, the epidermal growth factor receptor (EGFR) is often mutated or overexpressed. Downstream events resulting from constitutive activation of the EGFR pathway include transcriptional activation of antiapoptotic and proliferative genes, a key step in the transformation of global gene expression and initiation of tumor development (Uribe and Gonzalez, 2011). However, the characterization of specific molecular effectors that drive deregulated pathways also presents opportunities for therapeutic intervention. For example, the erbB2 gene (also known as HER2), a receptor tyrosine kinase belonging to the epidermal growth factor receptor (EGFR) family, is overexpressed in a subset of aggressive breast cancers and enhances several key pro-tumorigenic phenotypes (Moasser, 2007; Yu and Hung, 2000). However, a humanized monoclonal antibody (Trastuzumab) that targets the extracellular domain of the ErbB2 protein and downregulates its expression can inhibit tumor growth and enhance chemosensitivity (Shepard et al., 1991; Yu and Hung, 2000).

Beyond direct alterations in transcription factor expression or activity, gene expression pathways can also be deregulated by alterations in epigenetic and post-transcriptional mechanisms. Epigenetic profiles of tumor cells frequently differ greatly from their normal

tissue patterns (Feinberg and Vogelstein, 1983), yielding heritable changes in global gene expression that do not stem from changes in DNA sequence. Perhaps the best known epigenetic event associated with tumor cells is widespread hypomethylation of DNA, a state that alters transcription of nearby genes but also enhances chromosomal instability and mutation rates (Greger et al., 1989; Watanabe and Maekawa, 2010). Finally, cells employ a variety of mechanisms to control gene expression after transcription, largely through modulating the stability and/or translational potential of individual mRNAs or subpopulations thereof. The remainder of this review will principally focus on the regulation of mRNA turnover through the protein tristetraprolin, and how suppression of this mechanism can exacerbate pro-tumorigenic phenotypes and impede cellular senescence.

2. Post-transcriptional gene regulation and tristetraprolin

In eukaryotic cells, gene expression is normally tightly regulated through multiple control mechanisms. These regulatory systems are essential to ensure that gene products, whether they be protein or RNA, are maintained within levels appropriate for cellular growth, maintenance, function and apoptosis. An important determinant governing protein synthetic rates is the cytoplasmic concentration of their corresponding mRNAs, which is dependent on rates of both mRNA synthesis and degradation. The kinetics of gene transcription, pre-mRNA processing and nucleocytoplasmic transport are cumulatively responsible for mRNA synthetic rates and are subject to independent regulation. However, the cytoplasmic degradation rate of each mRNA is also tightly regulated, allowing the cell to quickly regulate transcript levels and hence their potential to program translation. Tight regulation of mRNA turnover kinetics is imperative for two principal reasons: [i] it can decrease the cell's response time between a transcriptional stimulus and its phenotypic output, and [ii] cells can vary the turnover rates of specific transcripts in response to diverse stimuli, thus providing a mechanism to rapidly modify protein production without modulating transcriptional activity (Guhaniyogi and Brewer, 2001; Hargrove and Schmidt, 1989).

2.1 AU-rich element (ARE)-mediated mRNA decay

AU-rich elements (AREs) are potent *cis*-acting determinants of cytoplasmic mRNA turnover in mammalian cells. The mRNA-destabilizing activity of AREs is essential for limiting cellular production of many clinically important gene products, including many oncogenes, cytokines, and inflammatory factors. AREs regulate mRNA turnover by interacting with ARE binding factors that impact transcript stability.

In mammals, AREs constitute a family of varied RNA sequences localized to the 3' untranslated regions (3'UTRs) of 8-10% of all mRNAs. In eukaryotic cells, the major mRNA decay pathway proceeds by 3'-5' shortening of the mRNA 3'-poly(A) tail, followed by rapid digestion of the mRNA body (Guhaniyogi and Brewer, 2001; Wilusz et al., 2001). The deadenylation phase of degradation appears to be rate limiting in the majority of cases, and the presence of an ARE generally accelerates mRNA turnover by increasing the deadenylation rate (Brewer, 1998; Chen and Shyu, 1995).

AREs from individual mRNAs are frequently conserved between species, yet there is astonishing diversity in the sequences of AREs between different transcripts. In general however, an ARE consists of a 40- to 150-nt U-rich region containing one or more repeats of the pentamer AUUUA. This motif has been found in an overlapping arrangement among AREs in mRNAs that encode inflammatory mediators and cytokines such as IL-3 and TNF α (Stoecklin et al., 1994; Xu et al., 1997). Interestingly, those AREs from proto-oncogene mRNAs such as *c-fos* and *c-myc* normally contain fewer AUUUA pentamers dispersed within a larger U-rich domain (Shyu et al., 1989). This varied population of ARE sequences found within the transcriptome is recognized by selected members of a group of *trans*-acting

factors collectively termed ARE-binding proteins (ARE-BPs) (Barreau et al., 2005; Wilson and Brewer, 1999). One such ARE-BP is AUF1, also known as heterogeneous nuclear ribonucleoprotein (hnRNP) D, which was first identified as an activity that could bind the ARE in *c-myc* mRNA and destabilize mRNAs containing this sequence (Zucconi and Wilson, 2011). The KH-type splicing regulatory protein (KSRP) is another example of an ARE-BP associated with accelerating decay of its mRNA targets, but also functions in the control of nuclear pre-mRNA splicing (Winzen et al., 2007). Other ARE-BPs including the neuronal-specific factors HuC and HuD, and their ubiquitously expressed family member HuR function to stabilize ARE-containing mRNAs, possibly by competing with the mRNA-destabilizing ARE-BPs for cognate substrate transcripts (Hinman and Lou, 2008). Finally, a distinct family of ARE-BPs typified by the proteins TIA-1 and the closely related protein TIAR do not appear to modulate mRNA decay kinetics directly, but rather control the translational efficiency of targeted transcripts (Anderson et al., 2004).

2.2.1 Tristetraprolin—Tristetraprolin (TTP; also known as ZFP36, TIS11, NUP475, and G0S24) is a 34-kDa member of the CCCH class of tandem zinc finger proteins and is expressed in a variety of tissues (Blackshear, 2002). TTP was first shown to interact with the ARE from TNF α mRNA, and its list of known and likely mRNA targets continues to grow (Lai et al., 2000). The strongest evidence of an mRNA-destabilizing role for this factor is observed in a TTP-deficient mouse model, where TNF α mRNA is significantly stabilized in macrophages, which leads to a significant elevation in circulating TNF α and contributes to symptoms of a systemic inflammatory syndrome (Carballo et al., 1998). Major phenotypes observed in TTP-deficient mice include medullary and extramedullary myeloid hyperplasia associated with cachexia, erosive arthritis, dermatitis, conjunctivitis, glomerular mesangial thickening, and high titers of anti-DNA and antinuclear antibodies (Taylor et al., 1996).

Current models indicate that TTP functions by binding selected ARE-containing mRNAs, then targeting them for destruction by recruitment of the deadenylase components Not1 and Caf1 (Sandler et al., 2011). However, TTP binding may also accelerate later stages of mRNA turnover, since co-purification experiments have identified components of mRNA 5'-decapping complexes (Fenger-Grøn et al., 2005) and factors responsible for both 5'-3' and 3'-5' degradation of the mRNA body (Hau et al., 2007) associated with cellular TTP. *In vitro* analyses of TTP binding to ARE fragments have shown that optimal binding requires an RNA sequence containing AUUUA or AUUUUA motifs flanked by additional uridylate residues (Brewer et al., 2004). However, given the sequence heterogeneity of AREs across the transcriptome it remains a challenge to explicitly identify the mRNA subpopulation that is subject to TTP-directed degradation.

2.2.2 Tristetraprolin as a potential tumor suppressor—Eukaryotic cell vitality is controlled by an array of extensively intertwined signaling networks and protein pathways. Neoplasia, in contrast, requires the deregulation of key cellular pathways leading to abnormal growth and malignancy. Given that ARE-containing mRNAs encode factors that are critical for cell growth, differentiation and apoptosis, it can be hypothesized that, as an ARE-BP, TTP may act as a key regulator controlling multiple cellular pathways that impact neoplastic development.

In 2003, Stoeklin *et al.* showed that TTP behaved as a tumor suppressor in a v-H-*ras*-dependent mast cell tumor model (Stoeklin et al., 2003). TTP expression in tumor cells decreased the abnormally high levels of IL-3 mRNA observed in these cells. Since proliferation of this tumor cell line is driven by an IL-3 autocrine loop, suppression of IL-3 synthesis by TTP dramatically slowed cell growth and significantly delayed tumor formation in murine xenografts. More recently, surveys of TTP expression in human tumors and cancer cell lines have yielded several findings suggesting that TTP may function as a tumor

suppressor in diverse neoplastic contexts (Brennan et al., 2009; Carrick and Blackshear, 2008). First, TTP expression was suppressed in many human cancers and cultured cancer cell lines relative to non-transformed tissues from many tissue sources. Second, TTP expression negatively correlated with tumor progression in breast and prostate cancers, and finally, breast cancer patients displaying low tumor TTP expression at tumorectomy showed significantly poorer disease-free survival than patients whose tumors expressed TTP at high levels. Interestingly, an independent study showed that genetic polymorphisms in TTP also correlated with reduced response to Trastuzumab in HER2-positive-breast cancer patients (Griseri et al., 2011). The mechanism(s) by which reduced TTP expression exacerbates tumorigenic phenotypes are not yet established; however, evidence suggests that the deregulation of many TTP target genes can promote diverse pro-tumorigenic phenotypes. In the next section of this review, we will discuss how interactions between TTP and known mRNA substrates can suppress several mechanisms associated with tumor development and progression, and how loss of TTP expression or function may deregulate these processes, thus promoting more aggressive neoplastic characteristics.

3. The impact of tristetraprolin on the hallmarks of cancer

3.1. Cancer-related inflammation

The role of inflammation during normal homeostasis is to protect the body against pathogens, injury, and in some cases tumors. However, the functions carried out by inflammatory cells and factors can also benefit tumor progression. The long term abuse of inflammatory mechanisms can alter gene expression patterns in cells, resulting in changes in cellular phenotypes that lead to dysplasia and cancer. Approximately 25% of all human cancers are attributed to the effects of chronic infections that cause inflammation and other chronic inflammatory syndromes (Hussain and Harris, 2007).

Extensive research during the past decade has revealed that several major cellular circuits link inflammation and cancer. Intrinsic and extrinsic mechanisms are accepted as the two basic pathways through which inflammatory and neoplastic conditions unite. The intrinsic pathway begins when neoplastic genetic events induce the expression of inflammation-related programs able to guide the establishment of an inflammatory microenvironment (Colotta et al., 2009). One such mechanism involves the extensive generation of cytokines and inflammatory factors which benefit the development and progression of tumors. These factors include TNF α , various interleukins, and growth and differentiation factors (Dranoff, 2004). In contrast, the extrinsic pathway is induced when chronic inflammatory conditions within normal tissue stimulate cancer development (Colotta et al., 2009). Chronic inflammation is diagnosed by: [i] sustained tissue damage, [ii] proliferation induced by damage, [iii] continuous tissue repair, and [iv] metaplasia, the reversible change of differentiated cell type (Lu et al., 2006; Nardone et al., 1999; Slack and Tosh, 2001). Infiltration of immune cells to a site of injury can prompt metaplasia progression to dysplasia, a condition in which chaotic proliferation results in atypical cell populations and neoplasia (Houghton et al., 2004; Prach et al., 1997). Whether by intrinsic or extrinsic means, cancer-related inflammation ultimately results in the infiltration of white blood cells, predominantly tumor-associated macrophages, the presence of inflammatory factors (cytokines such as TNF α , interleukin (IL)-1, IL-6, and chemokines such as CCL2 and CXCL8) and the occurrence of tissue remodeling and angiogenesis. Taken together, these additions to tumor microenvironment both aid in neoplastic transformation and in tumor progression (Colotta et al., 2009).

Early research with TTP revealed its importance in the balance of inflammatory response mechanisms. First, TTP knockout mice exhibit a severe chronic inflammatory phenotype (Taylor et al., 1996). However, TTP expression can be induced in response to selected pro-

inflammatory (*e.g.*, lipopolysaccharide) and anti-inflammatory (*e.g.*, glucocorticoids) stimuli (Carballo et al., 1998; Smoak and Cidlowski, 2006), supporting key roles for TTP in the control of inflammatory responses. More recent research identifying mRNA targets of TTP continues to support this model as many TTP substrate transcripts encode factors that are central to the control of inflammation and other pro-tumorigenic processes (Figure 1).

3.1.1 TNF α —Tumor necrosis factor α (TNF α) is a key pro-inflammatory cytokine. TNF α is synthesized as a transmembrane protein with a molecular mass of 26 kDa, but is then secreted as a soluble 17-kDa pro-peptide following cleavage by TNF α -converting enzyme. Circulating TNF α then mediates its effects through TNF α receptors I and II which promote expression of other inflammatory genes, principally through the activation of the NF- κ B and mitogen-activated protein kinase (MAPK) pathways (Wajant et al., 2003). Although activated macrophages are the primary source of TNF α , it can be produced by many other cell types including a wide variety of tumor cells. Emerging evidence has shown that TNF α is a major mediator of cancer-related inflammation and acts as a tumor promoting factor (Wu and Zhou, 2010). Constitutive TNF α production within the tumor microenvironment is a characteristic of many malignant tumors and is often associated with poor prognosis (Leek et al., 1998). In fact, an early report demonstrated that TNF α induces neoplasia 1000-fold more effectively than the chemical tumor promoters 12-*O*-tetradecanoylphorbol-13-acetate and okadaic acid (Komori et al., 1993). TNF α receptors are expressed on stromal and epithelial cells, facilitating cancer development by TNF α -regulated proliferation and survival of neoplastic cells. TNF α can also indirectly exert its effects through endothelial cells and inflammatory cells present within the tumor microenvironment (Wu and Zhou, 2010).

Regulatory mechanisms operate at virtually every step of TNF α expression, including post-transcriptional control events operating through an ARE located within the 3'UTR of TNF α mRNA. TTP binds directly to canonical high-affinity binding sites within the TNF α ARE (Brewer et al., 2004), and this interaction potentially destabilizes the TNF α transcript in cells (Carballo et al., 1998; Lai et al., 1999). In fact, the severe chronic inflammatory syndrome exhibited by TTP knockout mice is largely due to elevated circulating TNF α levels, since repeated administration of anti-TNF α antibodies largely suppresses these symptoms (Taylor et al., 1996). It is likely that downregulation of TTP expression in many cancers may favor tumor progression by elevating TNF α expression, thus enhancing the inflammatory state of the local tumor microenvironment.

3.1.2 COX-2—Cyclooxygenase 2 (COX-2; also known as prostaglandin-endoperoxide synthase 2) catalyzes the first step in the conversion of arachidonic acid into prostaglandins, most notably prostaglandin E₂ and related compounds, which are closely associated with the development of pro-inflammatory environments (Chizzolini and Brembilla, 2009; Williams et al., 1999). COX-2 is undetectable in most normal tissues as it is an inducible enzyme, only becoming abundant in activated macrophages and other cells at sites of inflammation. More recently, it has been shown to be upregulated in a wide variety of cancers and to have a central role in tumorigenesis (Simopoulos, 2002).

According to the American Cancer Society, colorectal cancer is the 3rd most common cancer in the US, and the second most common cause of cancer-related fatalities. Both abnormally high COX-2 expression and elevated levels of key prostaglandins are frequently found in colorectal tumor tissue (Williams et al., 1999). Interestingly, epidemiological studies show a 40-50% reduction in mortality for patients taking COX-2-inhibiting drugs such as aspirin (Aggarwal et al., 2006). These data support a critical role for COX-2 in the progression of some colorectal tumors, and elevated COX-2 expression has become a marker of poor patient prognosis in these cancers (Williams et al., 1999). High levels of COX-2 have also

been found in other cancers such as breast cancer, astrocytomas and head and neck squamous cell carcinoma (HNSCC) (Chan et al., 1999; Half et al., 2002; Rizzo, 2011; Shono et al., 2001; Tucker et al., 1999).

COX-2 expression is increased in the epithelium of many tumors, but more prominently in the tumor's stromal component. It is hypothesized that prostaglandins produced by stromally-derived COX-2 may promote tumor growth in a paracrine fashion by binding to members of a family of G protein-coupled receptors on nearby carcinoma cells. These receptors in turn activate downstream signaling events that induce expression of a host of gene products including factors that exacerbate the inflammatory state of the tumor microenvironment (Rizzo, 2011; Williams et al., 1999). In addition to promoting inflammatory signaling, COX-2 can also decrease apoptotic susceptibility. For example, COX-2 overexpression inhibits the cytochrome c -dependent apoptotic pathways as well as death receptor 5 expression and confers resistance to TRAIL-induced apoptosis in human colon cancer cells (Sun et al., 2002; Tang et al., 2002). Together, these properties have prompted consideration of COX-2 as a potent oncogene when deregulated and/or overexpressed in tumor or stromal tissue.

The COX-2 mRNA contains an ARE in its 3'UTR that associates with TTP in cancer cell models (Boutaud et al., 2003). In addition, TTP expression is suppressed in many adenocarcinomas when compared to normal colon tissue, and correlates with overexpression of COX-2 (Young et al., 2009). However, ectopic expression of TTP in these cells rescued cellular regulation of COX-2 production and attenuated cell proliferation, and furthermore antagonized enrichment of COX-2 levels mediated by the ARE-binding mRNA-stabilizing factor HuR (Cha et al., 2011). These data provide strong evidence that the reduced expression of TTP seen in many human colorectal cancers may be responsible in part for the increased expression of COX-2, which in turn can contribute to the aggressive characteristics and poor prognosis associated with these tumors.

3.1.3 IL-6—Interleukin 6 (IL-6) is a cytokine secreted by T cells and macrophages that stimulates inflammation and B cell maturation. Like many cytokines, IL-6 serves as a growth and survival factor that acts on pre-malignant cells and maintains a pro-tumorigenic inflammatory state. Association of circulating IL-6 with cognate cell surface receptors initiates signal transduction cascades that lead to activation of several transcription factors including STAT3 and NF- κ B (Grivennikov and Karin, 2011), resulting in downstream activation of their target genes. The NF- κ B pathway in particular is aberrantly activated in over 50% of cancers and renders premalignant or transformed cells resistant to apoptosis while also increasing their proliferation rates (Bollrath and Greten, 2009; Grivennikov and Karin, 2011). Elevated IL-6 expression can be detected in patient serum, and is linked to an increased risk of colorectal adenoma development (Kim et al., 2008). Consistent with this finding, genetic ablation of IL-6 in mice reduced both the size and number of colonic adenomas (Grivennikov et al., 2009). Expression of IL-6 in serum or tissue samples has been observed in many inflammation-based cancers, and generally correlates with poor patient prognosis (Grivennikov and Karin, 2011).

Studies from several labs have highlighted the contributions of TTP to the maintenance of cytokine homeostasis. For example, interferon-stimulated expression of TTP limits the induction of several pro-inflammatory cytokines, including IL-6, following exposure to bacterial lipopolysaccharide (Sauer et al., 2006). More recent work has shown that knockdown of TTP increases the stability of several cytokine mRNAs in head and neck squamous cell carcinoma cells, leading to elevated secretion of these factors (Van Tubergen et al., 2011). The relationship between TTP and IL-6 expression was also verified in TTP-deficient mice and embryonic fibroblasts from these animals, as each showed elevated IL-6

production relative to wild type models (Zhao et al., 2011). Taken together, these studies have established a strong link between TTP levels and expression of IL-6 as well as several other cytokines, leading to the appealing hypothesis that stimulation of TTP expression or activity might be a useful therapeutic strategy for suppressing multiple cytokines in inflammation-based cancers.

3.2 Sustained angiogenesis

Cells need to reside within 100 micrometers of capillary blood vessels in order to receive an adequate supply of oxygen and nutrients, highlighting the importance of organized and coordinated vascular development (Hanahan and Weinberg, 2011). However, observations of tumor vasculature have revealed blood vessel disorganization that is leaky and inefficient (Shchors and Evan, 2007). Despite the weakness of tumor-associated vessels, tumors cannot develop larger than 1-2 millimeters in diameter without induction of angiogenesis (Folkman et al., 1966). The exact timing when a tumor acquires sustained angiogenesis is uncertain, but there is supporting evidence for the existence of an “angiogenic switch”. At this point during tumor development, gene expression patterns shift to a profile which increases the production of blood vessels to provide nutrients and oxygen to the growing tumor (Hanahan and Folkman, 1996). New vessels can originate from pre-existing vessels in the tumor microenvironment or from circulating bone marrow-derived endothelial progenitor cells (BM-EPCs) (Avraamides et al., 2008; Lyden et al., 2011).

The normally quiescent vasculature can be activated to sprout new capillaries by a switching mechanism based upon changes in the relative balance of angiogenic inducers and inhibitors. In some tissues, the absence of angiogenesis inducers may keep the switch off, while in others the angiogenic inducers are present but held in check by higher levels of inhibitors (Hanahan and Folkman, 1996). It is clear that angiogenic inducers and inhibitors must be kept under tight regulation in order to control the switch, as deregulation would be extremely beneficial to a developing tumor mass. Several mRNAs encoding angiogenic inducers have been identified as TTP targets, and thus are aberrantly expressed in neoplastic cells where TTP expression is suppressed.

3.2.1 VEGF—Vascular endothelial growth factors (VEGFs) and their receptors are essential regulators of vascularogenesis, angiogenesis, and lymphangiogenesis (Holopainen et al., 2011). They function to create new blood vessels during development and after injury, create muscle following exercise, and new vessels to bypass blocked ones (Ferrara and Henzel, 1989). VEGF is a homodimeric glycoprotein and is the only known mitogen that specifically acts on endothelial cells (Millauer et al., 1994). All members of the VEGF family stimulate cellular responses by binding to tyrosine kinase receptors on the cell surface, causing them to dimerize and become activated through transphosphorylation (Holmes et al., 2007). The binding of VEGF to VEGF receptor 2 (VEGFR-2) triggers downstream signaling events that stimulate the permeability of existing blood vessels. This then forms a lattice network to direct the migration of endothelial cells and enhance chemotaxis of BM-EPCs (Baka et al., 2006; Batchelor et al., 2007).

The VEGF/VEGFR-2 signaling axis appears to be the most important regulator of blood vessel growth in tumors and is a key regulator of the angiogenic switch in cancer (Ferrara et al., 2007; Korsisaari et al., 2007). Early experiments showed that anti-VEGF monoclonal antibodies could inhibit growth of human tumors transplanted into nude mice (Ferrara et al., 2007). Also, elevated levels of VEGF mRNA correspond with the induction of VEGF found in most human tumors, and while hypoxia is thought to be the primary inducer of VEGF expression, several oncoproteins have also been shown to trigger its production (Kim et al.,

1993). As such, the regulation of VEGF expression is of primary importance to the restriction of tumorigenic angiogenesis.

Expression of TTP and VEGF was analyzed in resected colorectal cancer specimens by immunohistochemistry (Cha et al., 2011). TTP levels were low in cancerous cells, but high in non-malignant mucosa while VEGF levels correlated inversely. However, overexpression of TTP in cultured colon cancer cells markedly reduced VEGF mRNA levels (Cha et al., 2011). This and several other studies using diverse cell and/or tissue models have concluded that TTP inhibits VEGF production by destabilizing VEGF mRNA, and that reduced TTP expression may be responsible for the increased VEGF levels observed in a variety of human cancers (Brennan et al., 2009; Cha et al., 2011; Hacker et al., 2010; Lee et al., 2010a). This model is also consistent with data from xenograft studies that have shown decreased vascular development in tumors from cells that express TTP (Essafi-Benkhadir et al., 2007; Lee et al., 2010a). However, some recent studies have also described an alternative mechanism that can inhibit the ability of TTP to suppress VEGF expression in tumor cell models. TTP can be phosphorylated by stimulation of the ERK/MAPK pathway, but this event decreases TTP's mRNA-destabilizing activity, resulting in stabilization of VEGF mRNA and consequently an increase in VEGF expression (Essafi-Benkhadir et al., 2010). Similarly, hyperphosphorylated TTP, likely resulting from activation of the p38^{MAPK} pathway, was observed to be ubiquitously expressed in a panel of primary malignant glioma tissues concomitant with elevated VEGF levels. However, introduction of unphosphorylated TTP by conditional ectopic overexpression restored rapid VEGF mRNA decay kinetics (Suswam et al., 2008). Together, these examples show that functional TTP is a potent inhibitor of VEGF expression, with downstream implications for tumor vascularization. However, they also prompt new models whereby tumor cells can suppress the mRNA-destabilizing activity of TTP through protein post-translational modifications, in addition to previously described examples where TTP expression is inhibited in aggressive cancers.

3.2.2 HIF-1—Hypoxia-inducible factor 1 (HIF-1) is a heterodimeric transcription factor whose expression is highly regulated (Semenza, 2003). It coordinates metabolic adaptation to hypoxia and also directs oxygen delivery to cells via angiogenesis. HIF-1 is composed of two subunits, a constitutively expressed β subunit and an oxygen- and growth factor-regulated α subunit (Semenza, 2002). The HIF-1 α subunit is targeted by HIF prolyl-hydroxylase for degradation by hydroxylation during normoxic conditions, however during hypoxia the HIF prolyl-hydroxylase is inhibited, allowing the HIF-1 α subunit to accumulate (Semenza, 2004). Studies have also shown HIF-1 α induction in non-hypoxic conditions, as TNF α -induced NF- κ B appears to positively regulate HIF-1 α mRNA synthesis (vanUden et al., 2008).

There is also a growing body of research indicating that HIF-1 is a key player in carcinogenesis. In xenografts, tumor growth and angiogenesis are correlated with HIF-1 expression (Maxwell et al., 1997). In human cancers, HIF-1 α is frequently overexpressed as a result of intratumoral hypoxia and genetic alterations affecting key oncogenes (Semenza, 2002). As such it is not surprising that HIF-1 levels are elevated in the most common human cancers including those of the lung, prostate, breast, and colon, and are associated with increased patient mortality in several cancer types (Lopez-Lazaro, 2006). Finally, there is growing evidence suggesting that several oncogenic and tumor suppressor gene pathways are interconnected with the HIF-1 regulatory circuit (Vogelstein and Kinzler, 2004).

Similar to previous examples, control of HIF-1 α expression by TTP is also mediated by binding to the 3' UTR of HIF-1 α mRNA, and overexpressing TTP represses the induction of HIF-1 α synthesis normally observed under hypoxic conditions (Kim et al., 2010b). Conversely, siRNA-directed suppression of TTP expression stabilized HIF-1 α mRNA

during hypoxia, increasing accumulation of this transcript (Chamboredon et al., 2011). HIF-1 α mRNA contains eight putative TTP-binding motifs, suggesting that its expression may be extensively regulated through ARE-directed mechanisms, although reporter assays indicated that the two most distal AU-rich elements of HIF-1 α mRNA alone were sufficient for TTP-mediated repression (Chamboredon et al., 2011). By suppressing TTP expression, it is likely that tumor cells will enhance the amplitude of HIF-1-dependent transcriptional reprogramming associated with hypoxia, and thus more aggressively adapt to the nutrient restriction associated with tumor growth.

3.3 Tissue invasion and Metastasis

In general, primary tumors can be successfully treated with early detection, however once tumor cells acquire metastatic ability and travel to secondary sites patient survival declines. The initial primary tumor microenvironment plays an important role in this process of metastasis (Joyce and Pollard, 2009; Tlsty and Coussens, 2011). Properties of tumor cells, as well as the heterogeneity of the tumor cell population, can be influenced by hormones and cytokines but also by the specific population of stromal cells surrounding the tumor, since the cytokines and signaling molecules produced by stromal cells can activate several downstream effectors to enhance invasion and adhesion of tumor cells (Hiratsuka et al., 2006; Joyce and Pollard, 2009). While genetic and epigenetic alterations can promote progression of tumor cells to enter a metastatic state, the correct microenvironmental conditions are key for metastasis to occur (Shook and Keller, 2003). The process of tumor cell invasion requires departure from the original tumor site, survival during circulation through blood or lymphatic vessels, permeation into new tissue, and finally the acquisition of survival traits in the new environment (Joyce and Pollard, 2009; Mehlen and Puisieux, 2006). These challenges restrain potential metastatic cells so that less than 0.01% will survive out of the tens of thousands injected into circulation from a primary tumor every day (Fidler, 1970). Although many preventive and selective barriers exist which inhibit primary tumors from progressing to metastatic cases, this threatening diagnosis of metastasis is ultimately responsible for 90% of all deaths from human cancers (Sporn, 1996).

Some recent studies have provided evidence that suppression of TTP expression may contribute to enhanced invasiveness in selected tumor types. Surveys of prostate (Brennan et al., 2009) and breast cancer patients (Gebeshuber et al., 2009) showed significantly lower expression of TTP in primary tumors versus metastases. Similarly, in a cohort of breast cancer patients decreased tumor TTP mRNA levels were associated with increases in pathologic tumor grade, VEGF expression, and mortality from recurrent disease (Brennan et al., 2009). As mentioned previously, activating VEGF expression enhances tumor vascularization, which facilitates metastasis from primary tumor sites to distal tissues (Essafi-Benkhadir et al., 2007). Another study focused on TTP expression in cultured breast cancer cell models, finding that TTP levels were relatively high in non-invasive breast cancer cell lines like MCF10 when compared with the highly invasive MDA-MB-231 line, but also that ectopic expression of TTP significantly inhibited the invasiveness of MDA-MB-231 cells (Al-Shouhibani et al., 2010). Using a microarray strategy this group also identified a population of TTP-regulated mRNAs that are known to have prominent roles in breast cancer invasion and metastasis, and demonstrated that these transcripts were overexpressed in invasive cell lines. These data indicate that suppression of specific mRNA targets by TTP may play a protective role against cell invasiveness and metastasis, and also raise the possibility that loss of TTP expression may play a key role during the transition from a primary to a metastatic tumor.

3.3.1 miR-29a—MicroRNAs (miRNAs) are RNA molecules averaging 20 nucleotides in length that function as post-transcription regulators of gene expression. Within the context

of the RNA-Induced Silencing Complex (RISC), miRNA binding to complementary target mRNAs can repress translation of these transcripts or target them for degradation (Bartel, 2004; Bartel, 2009). Aberrant expression of miRNAs has been observed in many diseases and appears to contribute to tumorigenesis. In particular, some data implicate miRNA deregulation in metastatic progression (Calin and Croce, 2006; Gregory et al., 2008; Park et al., 2008). One example is miR-29a, which is dramatically enhanced in a cohort of metastatic human breast cancers relative to non-invasive tumors (Gebeshuber et al., 2009). Similarly, this miR was expressed at low levels in an H-Ras-V12-transformed mouse epithelial mammary cell model, but at much higher levels when these cells were directed through the epithelial-mesenchymal transition (EMT) by exposure to transforming growth factor- β (TGF- β). However, overexpression of miR-29a alone was sufficient to induce metastatic growths from epithelial cells in this model, supporting a causative relationship between miR-29a and EMT. TTP mRNA was identified as a functional target of miR-29a in these cells, and direct suppression of TTP expression using siRNA mimicked many features of miR-29a overexpression (Gebeshuber et al., 2009). Together, these exciting data suggest that the expression of many factors contributing to development of metastases may be controlled by a TTP-directed post-transcriptional gene regulatory circuit, but also provide evidence for a novel mechanism that might suppress TTP expression in a subset of aggressive cancers.

3.3.2 MMP1 and uPA/uPAR—The extracellular matrix (ECM) proteinases are divided into metalloproteinases, cysteine proteinases and serine proteinases (Mekki et al., 2009). Matrix metalloproteinase-1 (MMP1, also known as interstitial collagenase) is a zinc-dependent protease that degrades extracellular matrix proteins (Nagase et al., 1992). The protease acts as a molecular ratchet tethered to the cell surface, allowing it to move processively along collagen fibrils digesting collagen and other substrates during tissue remodeling (Saffarian et al., 2004). MMP1 levels are normally enriched in the tumor microenvironment and facilitate cell migration and invasion (Boire et al., 2005), and induction of MMP1 was identified as an important component of a gene expression signature associated with metastasis of breast cancer to the lung (Minn et al., 2005). Following a similar theme, the urokinase plasminogen activator (uPA) system encompasses a family of serine proteases involved in the degradation of the extracellular matrix and basement membranes. This degradation can impact cell adhesion and migration, and ultimately increases tumor cell invasiveness and metastasis (Dass et al., 2008; Mekki et al., 2009). High endogenous levels of uPA and its co-activating receptor (uPAR) are associated with advanced metastatic cancers, and the expression and activation of uPA plays an important role in tumorigenicity. Individual components of the uPA/uPAR system can be differentially expressed in cancer tissues compared to normal tissues, prompting substantial interest in the regulation of their expression (Dass et al., 2008). Microarray and ribonucleoprotein immunoprecipitation-based analyses of mRNAs regulated by TTP identified MMP1, uPA, and uPAR among a panel of metastasis-related genes that are normally suppressed by TTP-directed mRNA destabilization (Figure 1). Consistent with this finding, expression of these metastatic mediators is very high in cell models that do not express TTP, including the highly invasive breast cancer cell line MDA-MB-231 (Al-Shouhibani et al., 2010).

3.4 Evasion of apoptosis

Potential cancer cells undergoing proliferation become an even greater threat when natural cell death pathways, most notably apoptosis, are blocked (Kasibhatla and Tseng, 2003). After the initiation of apoptosis, cells acquire distinct physiological characteristics in just 30 to 120 minutes. Cellular membranes and cytoskeletons are broken down, the cytosol is expelled, chromosomes degrade, and the nucleus fragments. Proximal cells digest the

remains of the apoptotic cell within twenty-four hours (Wyllie et al., 1980). Typically, apoptotic pathways lie dormant until triggered by extracellular signals such as IL-3, Fas, and TNF α , or intracellular signals such as DNA damage, oncogene activation, or hypoxia (Evan and Littlewood, 1998). Apoptosis can occur through two principal mechanisms: (1) the extrinsic pathway, with the activation of cysteine aspartyl-specific proteases (caspases), or (2) the intrinsic pathway, with the release of cytochrome *c* and other pro-apoptotic molecules from mitochondria (Igney and Krammer, 2002; Locksley et al., 2001).

The multifaceted and sometimes redundant nature of the various cell death pathways normally presents a significant obstacle to tumor cell growth. Despite such hurdles cancer cells can evolve multiple ways of disrupting these pathways, which confer selective cellular advantages that drive continued tumor growth. While relatively little is known of mechanisms linking TTP to control of apoptosis, some data indicate that TTP can increase the sensitivity of cells to apoptotic cell death. For example, overexpressing TTP in some cell models including 3T3 fibroblasts and U251MG glioma cells induced apoptosis even in the absence of additional stimuli, but TTP also significantly enhanced cellular sensitivity to TNF α -induced apoptosis (Johnson et al., 2000; Suswam et al., 2008). In HeLa cells, restoration of TTP to physiologically relevant levels increased sensitivity to apoptosis induced by staurosporine, which principally activates the mitochondrial-directed pathway, but did not affect sensitivity to the DNA-damaging agent cisplatin (Brennan et al., 2009). These data suggest that TTP expression is associated with a pro-apoptotic state, but this may be limited to selected apoptotic triggering mechanisms and discrete subpopulations of cell types.

To date, no TTP substrate mRNAs have been clearly shown to link loss of TTP with resistance to intracellular apoptotic signaling. While a few mRNAs that encode apoptotic regulators are now known to be targeted and post-transcriptionally regulated by TTP, the functional significance of their repression by TTP remains unclear. For example, cIAP2 is a member of the inhibitor of apoptosis (IAP) gene family that is upregulated in many cancers and blocks activation of apoptotic cascades through several mechanisms (LaCasse et al., 2008). Canonical binding sites for TTP are located within the 3'UTR of cIAP2 mRNA, which can recruit TTP and target this mRNA for destruction (Kim et al., 2010a). By suppressing production of this apoptotic inhibitory factor, TTP would be considered to exert a pro-apoptotic effect in this context. However, TTP also binds and accelerates decay of the mRNA encoding Polo-like protein kinase 3 (Plk3), which normally functions to activate apoptosis in response to oxidative stress (Horner et al., 2009), suggesting an anti-apoptotic role for TTP in this system.

Cellular studies performed to date have provided some evidence that TTP-regulated expression of mRNA decay may impact cellular sensitivity to selected apoptotic stimuli, consistent with a general model whereby loss or diminution of TTP expression might help tumor cells evade apoptosis. Another intriguing possibility is that perturbations in TTP expression might contribute to chemotherapeutic resistance in tumors, as these agents frequently function by exploiting endogenous apoptotic mechanisms. However, while transcriptome-wide surveys of TTP substrate mRNAs (Al-Shouhibani et al., 2010; Lai et al., 2006; Stoecklin et al., 2008) have identified many potential targets through which TTP might influence apoptotic signaling, functional studies have yet to be performed on most of these TTP substrate candidates, and as such precise roles for TTP in the regulation of apoptosis remain undetermined.

3.5 Limitless replicative potential

A defining phenotype of cancer is accelerated cell growth which leads to the development of tumors. Deregulation of cell growth is achieved by cells that are able to: [i] supply the

metabolic needs for cell duplication, [ii] gain autonomy over normal growth signals, and [iii] develop insensitivity to growth inhibitory signals (Hanahan and Weinberg, 2011; Vogelstein and Kinzler, 2004; Weinberg, 1995).

A cancer cell must fulfill the bioenergetic and biosynthetic requirements of cell division to ensure that a viable daughter cell can be generated. Rapid induction of energy via ATP production and generation of other macromolecules allow tumor cells to meet the metabolic demands of proliferation (Vogelstein and Kinzler, 2004). Accordingly, enhanced glucose uptake and increased glucose metabolism have been observed in many cancer types. Glycolysis produces pyruvate which, due to the up-regulation of the enzyme lactate dehydrogenase in tumor cells, is mostly converted to lactate (Fantin et al., 2006). In non-transformed cells this is an inefficient form of metabolism, however, in tumor cells with excessive glucose uptake, ATP can be produced at a higher rate (Guppy et al., 1993). One of the earliest observations in cancer biology was the Warburg effect: the exploitation of glycolysis and inhibition of oxidative phosphorylation (Warburg, 1956).

Tumor cells also stimulate growth by acquiring the ability to manufacture extracellular growth signals and induce positive feedback signaling loops (Hanahan and Weinberg, 2011). For example, expression of platelet-derived growth factor (PDGF) is upregulated in many breast cancers. The enhanced levels of PDGF secreted from these cells can then bind and stimulate PDGF receptors, which in turn activate a host of intracellular pro-growth signaling and gene regulatory events (Orr et al., 1993). However, prospective cancer cells must also suppress their sensitivity to anti-proliferative signals, which normally hinder cell growth by inducing quiescence or by forcing entry into a postmitotic differentiated state (Hanahan and Weinberg, 2011). While limitless replicative potential was perhaps the earliest hallmark of cancer to be recognized, healthy tissue must undergo a profoundly complex transition in order for it to be achieved.

To date few studies have directly examined the role of TTP in controlling cell proliferation. In HeLa cells, restoring TTP to levels comparable to untransformed cervical tissue slowed cell proliferation by 50% (Brennan et al., 2009). This growth inhibitory activity required the RNA-binding activity of TTP, since a mutant form of the protein lacking this function had no effect on cell proliferation. TTP expression also exerted a negative effect on both proliferation and cell survival in the human glioma cell line U251MG (Suswam et al., 2008). Although little is known of the mechanism(s) by which TTP affects proliferation, additional studies have identified potential mRNA targets of TTP that encode factors intimately involved in control of cell cycle progression (Lee et al., 2010b; Sohn et al., 2010).

3.5.1 c-Myc—The *c-myc* (MYC) gene encodes a transcription factor that is believed to regulate transcription of as much as 15% of the human transcriptome (Gearhart et al., 2007). In healthy cells, c-Myc expression is induced by a host of mitogenic signaling pathways including Wnt, Notch, STAT, and selected receptor tyrosine kinases, and mediates its numerous biological effects by modulating transcription of its target genes (Larsson and Henriksson, 2010). A long-appreciated role for c-Myc is its ability to drive cell proliferation by inducing transcription from cyclin genes. However, c-Myc is also involved in facilitating cell growth, in part by enhancing production of ribosomal RNA, in the regulation of cell death by suppressing expression of the anti-apoptotic protein Bcl-2, and also plays roles in the control of cell differentiation and stem cell self-renewal (Denis et al., 1991).

The variety of cell cycle and growth stimulatory effects of c-Myc make it a very strong oncoprotein, which is potently upregulated in many types of cancers. In neoplasia, aberrant expression of c-Myc alters the transcriptional activity of myriad target genes, among which are many with oncogenic or tumor suppressive activities. However, beyond directly

reprogramming an extensive transcriptional regulatory network, c-Myc overexpression also indirectly promotes genomic instability through a wide range of mechanisms, including direct DNA damage resulting from increased generation of reactive oxygen species, gene amplifications, and mitotic spindle defects. Together these properties create a hypermutagenic environment that dramatically increases the likelihood of subsequent mutations in genes that control the cell cycle, apoptosis, and senescence (Neiman et al., 2008; Prochownik, 2008).

In many cancers, acquiring resistance to the antiproliferative effects of TGF- β 1 is crucial for malignant progression, and frequently results from constitutive induction of c-Myc (Pelengaris et al., 2002). A recent study examined the role of TTP on cellular responses to TGF- β 1 in liver cancer cells. TTP expression was suppressed in hepatocellular carcinoma cell lines compared with normal tissue, owing to methylation of a single CpG site within the TGF- β 1-responsive region of the TTP promoter (Sohn et al., 2010). Methylation at this site promotes binding of a transcriptional repressor, inhibiting both basal and TGF- β 1-induced transcription from the TTP gene. The resulting diminution of cellular TTP led to a significant increase in the stability of *c-myc* mRNA, consistent with previous observations showing that the *c-myc* transcript was targeted and destabilized by TTP (Marderosian et al., 2006), and consequent increases in c-Myc protein levels. This model was further supported by observations that methylation of the TGF- β 1-response element in the TTP promoter directly correlated with expression of c-Myc in tumor samples from hepatocellular carcinoma patients (Sohn et al., 2010). This example highlights how a specific epigenetic event that suppresses TTP expression can deregulate c-Myc production, thus facilitating resistance to a key antiproliferative signal during tumor progression.

3.5.2 LATS2—The LATS2 protein is a serine/threonine kinase with important roles in centrosome duplication and in the maintenance of genomic stability. LATS2 physically interacts with MDM2 to inhibit p53 ubiquitination and to promote p53 activation, which in turn permits cells to undergo p53-dependent G1/S arrest and avoid entering a tetraploid G1 state following damage to the mitotic spindle or centrosome dysfunction (Aylon et al., 2006). These functions classify LATS2 as a tumor suppressor and help to control cell homeostasis, and loss of LATS2 function is observed in a variety of tumor types including leukemia, breast and prostate cancer (Visser and Yang, 2010). However, recent work showed that LATS2 is significantly overexpressed in some nasopharyngeal carcinomas and is a poor prognostic indicator in patients. Furthermore, silencing LATS2 by siRNA in nasopharyngeal carcinoma cell lines actually inhibited growth suggesting a possible role for LATS2 in tumorigenesis (Zhang et al., 2010). Together, these examples highlight the incomplete nature of current models of LATS2 function, but also suggest that it is likely to have multiple activities including regulation of cell proliferation, cell death and cell migration, as well as broad governing roles such as transcriptional regulation and maintenance of genetic stability (Visser and Yang, 2010).

Recently, TTP was found to bind and destabilize LATS2 mRNA in the lung adenocarcinoma cell line A549, thus negatively regulating cellular LATS2 levels (Lee et al., 2010b). SiRNA-directed depletion of TTP in these cells enhanced LATS2 expression, which in turn modestly inhibited cell proliferation. This effect was abrogated if LATS2 expression was also suppressed. These data prompt a curious possibility that TTP could function as a pro-tumorigenic factor in this cellular context, by inhibiting expression of a tumor suppressor.

4. The role of tristetraprolin in senescence

4.1 Senescence

Senescence is a mechanism employed to prevent cell growth past a certain number of replicative cycles, known as the Hayflick's limit (Lyden et al., 2011). This constraint varies depending on cell type but is normally about 40 to 60 replications based on critical telomere length. Morphological and biochemical alterations of senescent cells include flattening of cytoplasm and increased granularity, induction of β -galactosidase and alterations in gene expression patterns (Holopainen et al., 2011).

Prospective tumor cells that acquire mechanisms to circumvent senescence and undergo limitless replication gain a tremendous growth advantage. Frequently, cancer cells evade senescence by reactivating the telomerase enzyme which maintains telomeres at chromosome ends (Kelland, 2007). Also, fibroblasts with defective pRb and p53 proteins can divide through generations past normal replication limits (Beausejour et al., 2003).

4.2 Tristetraprolin induces senescence

Relatively few studies have examined the functional relationship between TTP and cellular senescence. In a survey of human tissues, levels of TTP protein and the relative proportion of cells expressing TTP decreased as a function of age in several cell types, including those of the immune, nervous, and muscular systems, suggesting that TTP does not promote cellular senescence in these tissues (Masuda et al., 2009). However, B lymphocytes from aged mice typically exhibit elevated levels of TTP relative to cells from young mice (Frasca et al., 2008). Higher TTP in the older cells binds and destabilizes the mRNA encoding the transcription factor E47, an important regulator of immunoglobulin class switching. Cultured cell-based studies reported to date also generally support a pro-senescence role for TTP. For example, in human diploid fibroblasts, cells approaching senescence exhibited a substantial elevation in TTP expression (Masuda et al., 2009).

A potential mechanism linking TTP to cellular senescence was developed using human papillomavirus (HPV)-transformed cervical cancer cells as a model. HPV integration into the human genome enables the expression of viral proteins such as E6 and E7 which promote HPV-induced carcinogenesis (Kisseljev et al., 2008; Stanley et al., 2007). Specifically, these proteins prevent infected cells from entering senescence by maintaining a dormant p53 pathway and elevated telomerase activity (Gallouzi, 2009). E6 forms a complex with the E6-associated protein (E6-AP), a cellular E3 ubiquitin ligase, which then binds p53 and targets the protein for ubiquitin-dependent degradation (Huibregtse et al., 1991; Scheffner et al., 1990). However, TTP can bind and destabilize E6-AP mRNA, and the resulting diminution of E6-AP protein levels stabilizes the p53 protein, allowing it to accumulate in the cell (Sanduja et al., 2009). As a result, HPV-positive HeLa cells that overexpress TTP exhibit the growth arrest and β -galactosidase activities typical of senescence. While post-transcriptional suppression of E6-AP expression can account for the senescence-promoting potential of TTP in this model system, it remains unclear whether other TTP substrate mRNAs encode factors that limit senescence more generally.

5. Oncogenic mechanisms that suppress tristetraprolin

Several studies have shown that TTP expression is suppressed in many cancers, and that there is frequently an inverse correlation between TTP levels and tumor aggressiveness. Following from these findings, numerous groups have examined the mechanisms linking loss of TTP expression to tumor development, from perspectives of both global cellular phenotypes and also characterization of deregulated TTP substrate mRNAs (described above). However, among the fundamental questions that remain unanswered are: [i] what

mechanism(s) are responsible for suppressing TTP expression in cancer? and [ii] is TTP suppression a critical step in the transition from healthy into neoplastic tissue, or in the development of metastases?

To date, a few published works have provided insights into cellular mechanisms that can diminish TTP expression and/or activity in specific cellular contexts. One example described above details an epigenetic mechanism that can suppress TTP transcription. A single methylated CpG site in TGF- β 1 response element contained within the TTP gene promoter is sufficient to silence TTP expression in a hepatocellular cancer cell line (Sohn et al., 2010). Furthermore, this CpG site was frequently methylated in tumors from hepatocellular carcinoma tumors relative to normal liver tissue. A second mechanism involves post-transcriptional suppression of TTP expression by the microRNA miR-29a, which is overexpressed in metastatic human breast cancers relative to non-invasive tumors, and is sufficient to induce invasive characteristics in an epithelial murine mammary cancer cell model (Gebeshuber et al., 2009). In a broader sense, the roles of other cellular ARE-BPs could also play a role in buffering or enhancing perturbations in TTP expression, as extensive post-transcriptional cross-talk exists between these factors. Many ARE-BPs are encoded by mRNAs that contain ARE-like sequences in their 3'UTRs, which can bind and be regulated by different subsets of the cellular ARE-BP population (Pullman et al., 2007). Although ARE-BP binding to TTP mRNA has not been rigorously characterized, the TTP transcript also contains a 3'UTR-localized ARE, which can bind TTP protein in an apparent autoregulatory loop (Brooks et al., 2004; Tchen et al., 2004). Finally, TTP expression and/or activity can be regulated post-translationally through hyperphosphorylation by the p38^{MAPK} and ERK MAP kinase pathways. In melanoma cells, constitutive ERK activity suppresses TTP levels by targeting the protein for degradation by the proteasome (Bourcier et al., 2011). By contrast, experiments in bone marrow-derived macrophages suggest that TTP phosphorylation by the p38^{MAPK}-activated protein kinase 2 (MK2) actually increases the stability of the TTP protein but reduces its affinity for ARE-containing mRNAs (Hitti et al., 2006). A later study using HeLa cells provided evidence that phosphorylation of TTP by MK2 could also inhibit its ability to degrade mRNA by blocking recruitment of the CAF1 deadenylase enzyme (Marchese et al., 2010). In summary, recent studies have identified a wide variety of potential mechanisms that can regulate the expression or activity of TTP, and have described examples where some of these events have been subverted to suppress TTP in specific cancer cell models.

6. Future directions

Owing to the complex and varied cellular mechanisms responsible for initiation and development of cancers, disease presentation, optimal treatment strategies, and prognosis can vary widely between patients. While activation of specific oncogenes is a common theme, their effects on intracellular signaling pathways or specific gene expression events ultimately direct dramatic reprogramming of gene regulatory networks in transformed cells. This oncogenic “amplification response” provides a means whereby a small number of mutated genes can impact the diverse array of cellular phenotypes characteristic of cancer. Perturbation of post-transcriptional gene regulation by altering the expression or activity of ARE-BPs, including TTP, is now emerging as a powerful method for reprogramming cell homeostasis on a vast scale.

A growing body of research has documented the suppression of TTP in tumors, many of the mRNA targets that this factor recognizes, and the effects of deregulated TTP expression and/or activity on specific tumor cell properties. These largely mechanistic studies have focused on how loss of TTP can enhance tumorigenicity, to which additional questions are now being added: How is TTP expression lost during tumorigenesis? How does diminishing

or deactivating TTP give tumor cells an advantage over TTP expressing cells? Can TTP ever play a pro-tumorigenic role? However, added to this we can consider new research questions that apply our growing understanding of the relationships between TTP and tumor progression to more directly benefit patients. First, does TTP have utility as a diagnostic biomarker in cancer? To resolve this, we need to understand how the consequences of TTP suppression vary among different cancer types. Also, do patients with high versus low tumor TTP levels respond differently to conventional therapeutic strategies? If so, then assessment of TTP expression in tumor biopsies could help inform optimal therapeutic strategies on a patient-by-patient basis. A second and very exciting downstream question will be whether targeted restoration of TTP expression has potential as a therapeutic strategy in cancer. To address this, we will need to start by asking: Can TTP expression be selectively restored in tumors *in vivo*? How does restoration of TTP affect tumor progression *in vivo*? Current understanding of the roles of TTP in senescence and aging are much less developed, but most models suggest that elevated TTP expression correlates with induction of senescence. By this logic, could targeted suppression of TTP be useful in combating some features of aging? Given how quickly our appreciation of the basic biological mechanisms of TTP has developed, rapidly followed by emerging insights into its involvement in many processes associated with tumor progression and new roles in senescence, the new frontier will be to apply this knowledge to new tools and strategies that directly impact human health and disease.

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Highlights

1. Tristetraprolin (TTP) binds mRNAs containing AU-rich elements (AREs) and targets them for rapid mRNA decay.
2. TTP expression and/or activity are potently downregulated in many human cancers, particularly advanced and aggressive cancers.
3. TTP suppresses the expression of many mRNAs encoding factors that control diverse aspects of tumor development and senescence; as such, loss of TTP in cancer reprograms a gene regulatory network that exacerbates many tumorigenic phenotypes.

Figure 1.

A model for coordinated control of pro-oncogenic post-transcriptional gene regulatory networks by TTP. The listed mRNAs encoding oncogenic factors and evidence for TTP mediated post-transcriptional regulation of these transcripts is described within the text.