Post-transcriptional regulation in cancer progression

Microenvironmental control of alternative splicing and translation

Michael Jewer · Scott D. Findlay · Lynne-Marie Postovit

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Abstract The microenvironment acts as a conduit for cellular communication, delivering signals that direct development and sustain tissue homeostasis. In pathologies such as cancer, this integral function of the microenvironment is hijacked to support tumor growth and progression. Cells sense the microenvironment via signal transduction pathways culminating in altered gene expression. In addition to induced transcriptional changes, the microenvironment exerts its effect on the cell through regulation of post-transcriptional processes including alternative splicing and translational control. Here we describe how alternative splicing and protein translation are controlled by microenvironmental parameters such as oxygen availability. We also emphasize how these pathways can be utilized to support processes that are hallmarks of cancer such as angiogenesis,

proliferation, and cell migration. We stress that cancer cells respond to their microenvironment through an integrated regulation of gene expression at multiple levels that collectively contribute to disease progression.

Keywords Tumor microenvironment · Alternative splicing · Translation · Cancer progression

Introduction

Cellular phenotypes are tightly regulated by molecular signals such as oxygen gradients and growth factors that collectively constitute the microenvironment. This microenvironment provides the extracellular cues for growth, differentiation, and death throughout the lifespan of a cell. However, if a cell accumulates sufficient mutational and epigenetic changes, it may become aberrantly insensitive to anti-proliferative cues, or itself contribute pro-proliferative signals to the microenvironment. Both of these aberrant processes can result in uncontrolled cell division and ultimately cancer. Thus, normal physiology depends on dynamic interplay between a cell and its microenvironment, with disruption potentiating tumor initiation and progression toward metastatic disease.

In cancer, an altered microenvironment provides a context similar to that of early embryonic development, marked by the presence of pro-proliferative growth factors and hypoxic growth conditions. Consequently, some tumor cells can acquire embryonic-like phenotypes characterized by an increased potential for proliferation and survival. Classically, molecular components and changes mediating such processes have been assessed in terms of epigenetic alterations potentiating changes in gene transcription (Postovit et al. 2008). However, it is now becoming apparent that numerous levels of post-transcriptional regulation are also integral to the tumor cell response to the microenvironment. This review will focus on alternative pre-mRNA splicing and translational control as

Summary Classically, the influence of the tumor microenvironment on the regulation of cellular phenotype has been assessed principally in terms of changes in gene expression at the level of transcription. Here, we detail evidence of an integrated response that also involves control of alternative splicing and protein translation, collectively contributing to tumor progression.

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M. Jewer · S. D. Findlay · L.-M. Postovit (✉)
Department of Anatomy & Cell Biology, The Schulich
School of Medicine and Dentistry, Western University,
438 Medical Science Building,
London, ON N6A 5C1, Canada
e-mail: Lynne.Postovit@schulich.uwo.ca

two major points of post-transcriptional regulation mediating tumor progression in response to the microenvironment. Analogous to “turning on” and “turning off” genes through transcriptional regulation, control over alternative splicing dictates which transcript (often with strongly divergent or opposing functions) will be expressed from a single gene. Control over translation dictates which transcripts are translated under different contexts, often independent of transcript abundance, as the global correlation between transcript and corresponding protein is only moderate (e.g. Schwanhausser et al. 2011) (Fig. 1).

Alternative splicing

Mechanisms of alternative splicing (AS) exist for many genes whereby different combinations of exons within a single pre-mRNA can be included in distinctly processed transcripts that serve as substrates for translation. Recent genome-wide analyses suggest that AS might affect as many as 95 % of multi-exon human transcripts (Wang et al. 2008). The relative positions of utilized 5' donor and 3' acceptor splice sites are used to classify different types of AS events as cassette alternative exon, mutually exclusive exon, alternative 5' splice site, alternative 3' splice site, or intron retention (Fig. 2). This newfound appreciation of the ubiquity of AS suggests there are numerous alternatively spliced transcript isoforms yet to be characterized.

Fig. 2 Major types of alternative splicing. *Orange bars* represent constitutive exons always included in the processed mRNA transcript. *Blue bars* represent alternative exons, and *grey bars* represent introns. *Diagonal lines* join splice donor sites at the 5' end of an intron and splice acceptor sites at the 3' end of an intron, flanking the intronic sequence to be spliced out. Splicing sites along the top of the transcript denote one possible transcript, while those along the bottom denote the other isoform for these simple cases

How is splicing altered in cancer?

As is true for gene transcription, patterns of AS also differ among adult tissues such as brain, skeletal muscle, breast, liver, and colon. For example, only 2 % of processed transcripts for the tropomyosin 1 (alpha) gene (TPM1) contained exon 2 in heart tissue, while in skeletal muscle

Fig. 1 A schematic of how fate decisions are regulated in part by microenvironmental factors. The *large blue horizontal arrow* represents how differentiated cells can become dedifferentiated during tumorigenesis. This process involves cellular responses to the microenvironment that include changes in transcription, as well as

post-transcriptional processes such as alternative splicing and translation. An example in which expression of one protein isoform is increased (*green arrow*) and the alternatively-spliced isoform is decreased (*red arrow*) is shown

such transcripts accounted for 95 % of all TPM1 spliced (Wang et al. 2008). It follows that AS is tightly regulated over the course of development, and that specific patterns of splicing must be maintained in adult tissues to preserve distinct cellular identities and functions. Unsurprisingly, much like transcriptional regulation of gene expression, control over AS is commonly deregulated in cancer and has been the focus of several excellent reviews (David and Manley 2010; Fackenthal and Godley 2008; Srebrow and Kornblihtt 2006; Watermann et al. 2006).

Altered AS can contribute to cancer progression in different fashions. First, mutations or polymorphisms within a splice site motif such as a 5' splice donor or a 3' splice site acceptor can result in altered splicing of transcripts transcribed from the affected locus. For example, in breast and ovarian cancer, mutations in the tumor suppressor breast cancer 1, early onset (BRCA1) often disrupt constitutive splice sites, leading to the production of isoforms that cannot translate functional protein (Thomassen et al. 2011). Second, the regulation of particular AS events can become generally disrupted due to widespread genetic, epigenetic, and post-transcriptional changes associated with malignant transformation and spread. Notably, such disruption is not necessarily directly related to mutation status of the affected transcript(s). For example, exposure of primary epithelial cells to insulin growth factor 1 (IGF1) and tumor necrosis factor alpha (TNF- α) promotes increased splicing of pro-angiogenic vascular endothelial growth factor A (VEGFA or VEGF) VEGF_{xxx} isoforms relative to the anti-angiogenic VEGF_{xxx}b isoforms. Thus any ectopic activity of these growth factors in cancer likely contributes to enhanced tumor angiogenesis in part through a specific AS change (Nowak et al. 2008).

Interestingly, the potential also exists for alternative splicing to modulate protein structure through the inclusion or skipping of exon(s), in cases where each exon encodes for a distinct protein domain. Indeed this is the case for the CCN proteins, with characteristic signal peptide (SP), insulin growth factor binding protein-like (IGFBP), Von Willebrand type C repeat (VWC), thrombospondin (TSP1) and C-terminal cysteine knot (CT) domains each encoded by individual exons. Alternatively spliced transcripts of this nature have been identified for CCN1, CCN3, and CCN4 (Perbal 2009). These transcript variants have been identified in pathologies such as scirrhous gastric carcinoma, cholangiocarcinoma, hepatoma, Ewing's tumors, Wilm's tumors, and human breast tumors. How these transcripts may contribute to cancer pathology is the focus of an excellent review (Perbal 2009). One interesting example of such splicing is found in a study by Tanaka and colleagues focused on CCN4 (WISP1), a protein implicated in fibrosis. RT-PCR of gastric carcinoma tissue samples revealed a novel WISP1 transcript, designated WISP1v. This smaller transcript variant was detected in the majority of scirrhous type gastric carcinoma (86 % of

samples) associated with a poor prognosis, but in few of the non-scirrhous gastric carcinoma samples (15 %), and patient-matched normal adjacent mucosa samples (Tanaka et al. 2001). Sequence analysis revealed this transcript resulted from skipping of exon 3 which encodes the VWC domain in full length CCN transcripts. Transfection of NIH3T3 fibroblasts with either WISP1 or WISP1v revealed enhanced cellular transformation, more rapid cellular growth, as well as increased migration of gastric carcinoma cells in co-culture, for fibroblasts transfected with the novel WISP1v relative to those transfected with canonical WISP1 (Tanaka et al. 2001).

CCN1 (Cyr61) offers a mechanistically distinct example of how a change in AS can promote tumor progression. CCN1 promotes cell proliferation, migration, and adhesion (Kireeva et al. 1996), as well tumour angiogenesis (Babic et al. 1998) and is of great interest in breast cancer, where its expression is positively correlated with both disease stage and positive lymph nodes (O'Kelly et al. 2008). Hirschfeld and colleagues demonstrated the existence of a CCN1 transcript variant wherein intron 3 is retained in the processed transcript. Interestingly, this isoform was shown to be the predominant CCN1 transcript in noncancerous normal tissue, with little canonical CCN1 detected. However, in patient-matched samples of primary adenocarcinoma of the breast, roughly equal levels of the intron-retaining and canonical intron-skipping transcripts were observed (Hirschfeld et al. 2009). Since there are two stop codons within the third intron and in the translational reading frame, the authors hypothesize that the intron-retaining isoform likely triggers nonsense-mediated decay (NMD), a process that contributes to transcript degradation and limited protein expression, typically when stop codons are present more than 50 nucleotides upstream of an exon-exon junction (Gardner 2010). Thus, alternative splicing actively maintains CCN1 expression at sub-pathogenic levels in normal adult tissue. In cancer, this regulatory mechanism is lost, leading to the productive translation of the canonical tumorigenic isoform (Hirschfeld et al. 2009). Although NMD is the most likely result of intron-retaining CCN1 splicing, the authors did not consider the possibility that the intron-retaining isoform of CCN1 mRNA might translate to a protein product in which only the SP, IGFBP, and VWC domains are present due to the premature termination codon upstream of the fourth exon. The existence of such an isoform would be exciting and is worthy of exploration, as has been mentioned previously (Perbal 2009).

Beyond CCNs, the global collection of AS events altered in cancer contribute to many pathological processes such as evasion of anti-tumor immunity (largely concerning apoptosis), alterations in metabolism, invasion and metastasis, epithelial to mesenchymal transition (EMT), angiogenesis, and translation of oncogenic protein isoforms (reviewed in David and Manley 2010). In an interesting metabolic example, the splicing of an embryonic form of pyruvate kinase known as

PKM2, allows cancer cells to undergo aerobic glycolysis, a process thought to be essential in meeting biosynthetic demands in a highly proliferative tumor. In contrast, normal differentiated cells perform aerobic glycolysis at much lower rates owing to splicing of the PKM1 isoform (Christofk et al. 2008). Dysregulation of AS is widespread in cancer, occurring in pathways related to the original six (and now more numerous) “hallmarks of cancer” described by Hanahan and Weinberg (Hanahan and Weinberg 2000, 2011), and thus may present opportunities for therapeutic intervention and novel prognostic biomarker identification for specific cancers (Dales et al. 2010; Huang et al. 2007).

Translational control

As the greatest expenditure of energy in the cell is protein synthesis, it is fitting that translation is tightly regulated. The rate limiting step of translation and the point of tightest regulation is initiation (Gingras et al. 2001). Initiation requires the coordination of multiple proteins, named eukaryotic initiation factors (eIFs). eIFs are required for recognition of the m7GpppN cap, recruitment of the ribosome, and ribosomal positioning at the translational start site (Mamane et al. 2006). Translation initiation is reduced during conditions of stress. eIFs like eIF4E and eIF2 α have their function inhibited by stress pathways like the mTOR pathways and stress signals from the ER. Because of the central role of translation in cellular physiology, it is not surprising that it is critical in development, differentiation, cell cycle progression, and apoptosis (Gingras et al. 2001). Alterations to the proteome that initiate from changes in translation occur faster than changes that require transcription by circumventing the need to produce and export transcripts from the nucleus (Gingras et al. 2001). The rate of the translational response and the extent to which it can change expression patterns and conserve energy, gives translation a central role in responding to the microenvironment.

How is translational control altered in cancer?

Two themes permeate the role of translation in cancer. The first theme is that in times of stress, cancer cells will limit translation to a subset of proteins that broadly promote survival, consequently causing a more aggressive cancer. The second theme is that increases in the proteins required to initiate translation, eIFs, release important modulators of the cell cycle leading to uncontrolled growth (Li et al. 2003, 2004b; Mamane et al. 2006; Tan et al. 2000).

Microenvironmental factors are primarily responsible for activating the translational stress response. Cells in internal regions of a tumor often lack the local vasculature necessary to deliver nutrients and oxygen. Such cells become stressed,

triggering a decrease in global rates of translation in order to maintain energy homeostasis. One of the most potent mediators of this conservation is hypoxia (Liu et al. 2006; Wouters and Koritzinsky 2008). Two mechanisms are responsible for ensuring the decrease in translation observed during stress. First, phosphorylation of eIF2 α in response to endoplasmic reticulum stress causes eIF2 α to remain bound to GDP; In this inactive state, recognition of the start site of translation is prevented. Multiple pathways can inactivate mammalian target of rapamycin (mTOR) which leads to the dephosphorylation and activation of eIF 4E binding protein (4E-BP), a translational repressor. 4E-BP binds to eIF4E preventing the formation of the cap-binding complex. Without eIF4E the translational complex cannot identify the 5' cap (Young et al. 2008). Under stress, cap-dependent translation is suppressed. This process conserves valuable energy while inducing an adaptive stress response, such that transcripts encoding proteins required for survival can still recruit ribosomes by means of internal ribosome entry site (IRES) sequences in their 5'UTRs (Young et al. 2008).

Various changes in translation initiation have been demonstrated to have broad effects on pathogenicity. Early work showed that many individual eIFs are up-regulated in cancer and that these changes correlate with cancer progression (Eberle et al. 1997; Kerekatte et al. 1995; Li et al. 1997; Miyagi et al. 1995). These findings were expanded by the use of global microarray analyses of multiple tumor types which found a strong correlation between the amount of eIF4E and the expression of both the pro-angiogenic factor VEGF, and the cell cycle protein cyclin D1 (Yang et al. 2007). Other eIFs have been shown to be deregulated in cancer and have been reviewed extensively (Yin et al. 2011).

The activity of mTOR has also been correlated with malignancy in melanoma and prostate cancers (Karbowiczek et al. 2008; Pópulo et al. 2010). Furthermore, many cancers have been shown either clinically or experimentally to be responsive to mTOR inhibitors (summarized in Mamane et al. 2006). Increased mTOR activity disrupts proper control of translation by inactivation of 4E-BP. An increase in the phosphorylation of 4E-BP has been clearly correlated with survival in patients with cancers such as melanoma (O'Reilly et al. 2009). Inactivation of 4E-BP via phosphorylation prevents it from binding eIF4E, maintaining a high level of cap-dependant translation. Similarly, over-expression of 4E-BP has been shown to reduce tumorigenesis and growth (Rousseau et al. 1996). 4E-BP can even compensate for transformation caused by increased levels of eIF4E (Avdulov et al. 2004). Further evidence for the importance of translational regulation was found by expressing constitutively active 4E-BP mutants in U2OS osteosarcoma cell lines. Cell size and cell cycle progression could be regulated by the 4E-BP mutant, as the mutant phenotypes mimicked those caused by treatment with rapamycin (Fingar et al. 2002, 2004).

Regulation of translation is important for maintaining the balance between apoptotic and cell survival signals (Mamane et al. 2006). eIF4E increased chemoresistance and decreased apoptosis in fibroblasts with constitutively expressed c-myc, primarily through increasing the rate of cyclin D1 translation (Tan et al. 2000). Li and colleagues showed that the reduction of eIF4E decreased cytochrome c release and endoplasmic reticulum-mediated apoptosis, implicating the increased translation of Bcl-X_L as part of the mechanism (Li et al. 2003). Similar experiments utilizing antisense oligonucleotides to knock down eIF4E in MDA-MB-231 cells decreased another Bcl protein, Bcl-2 (Graff et al. 2007). The many roles eIF4E has in the progression of cancer, the frequency of its up-regulation, and its transforming potential in NIH 3T3 cells have identified it as a proto-oncogene (Lazaris-Karatzas et al. 1990). eIF4A1, a well studied RNA helicase, has also been implicated in several cancers including melanoma, hepatocellular carcinoma, and early stage non-small cell lung cancer (Eberle et al. 1997; Shuda et al. 2000; Wang et al. 2002). To assess the direct effect of eIF4A1 on Bro and SKM13 melanoma cell lines, each line was transfected with vectors including inducible eIF4A1 antisense RNA. Using thymidine incorporation rates to measure proliferation, Eberle et al. demonstrated that eIF4A1 expression is positively correlated with proliferation in melanoma cell lines. The mechanism for this increase in proliferation is unknown but similar experiments evaluating the effects of expression of another eIF, eIF2 α , demonstrated an up-regulation of cyclin D1 (Rosenwald et al. 2003). Thus, altered translational regulation can greatly affect proliferation.

Signaling from the microenvironment

Combinatorial induction of AS and translational responses

A major hallmark of cancer is the acquired ability to sustain growth and survival while adapting to alterations in the microenvironment. This is achieved largely through aberrations in key signaling pathways that link extracellular growth factors with intracellular signaling cascades that modulate gene expression and other cellular processes. Indeed, AS and translational control are susceptible to dysregulation via these dysfunctional signaling pathways, and represent additional mechanisms through which tumor growth and survival are achieved.

Microenvironments affecting splicing

A role for tumor-associated signaling in the regulation of AS in developmental pathways is emerging. Notch is a transmembrane receptor that binds members of the Delta-like and Jagged families of ligands expressed on the surface of adjacent cells.

Ligand binding results in cleavage of the Notch Intracellular Domain (NICD) and subsequent activation of CSL family transcription factor complexes, affecting transcription of genes such as *Myc*, *Cyclin D3*, and *p21*, thus affecting decisions pertaining to cell fate. In addition to direct canonical transcriptional targets, Notch signaling can also indirectly affect AS. For example, constitutive Notch signaling is known to promote leukemogenesis in T cell acute lymphoblastic leukemia. One mechanism by which this is achieved is through Notch-dependent increased splicing of a non-DNA binding and dominant negative isoform of the transcription factor Ikaros, also implicated in leukemogenesis (Bellavia et al. 2007).

Extracellular ligands of the transforming growth factor-beta (TGF- β) superfamily help establish the body plan during development by initiating signaling cascades that regulate differentiation, migration, and proliferation. Generally, TGF- β signals through serine/threonine kinase receptors at the cell surface resulting in subsequent activation of specific SMAD proteins that mediate changes in gene expression through interaction with various transcription factors. TGF- β signals affect splicing of several transcripts in normal cellular contexts (Chang et al. 2004; Martin et al. 2006). In cancer, regulation of splicing events by TGF- β is consistent with the notion that this ligand can act as both a tumor suppressor as well as key component in the progression of cancers. TGF- β has been shown to potentiate the fibroblast growth factor receptor (FGFR) splice switch associated with cancer progression in a mouse mammary epithelial model of EMT (Shirakihara et al. 2011), but also preferentially splices anti-angiogenic VEGF_{xxx_b} isoforms which are down-regulated during the progression of many tumors (Nowak et al. 2008).

Signaling converges on “splicing hubs”

The effects of growth factors and other components of the microenvironment on AS are mediated by intracellular signaling cascades (Li et al. 2004a; Stamm 2002). These cascades ultimately affect splicing regulatory proteins such as members of the SR and heterogeneous nuclear ribonucleoprotein (hnRNP) families (David and Manley 2010; Lynch 2007). Two “splicing hubs” through which a multitude of cascades converge to regulate AS have been identified as hnRNP K and Sam68. hnRNP K has been shown to bind pre-mRNA splicing enhancers and silencers, with direct phosphorylation of hnRNP K by Src-kinases, Protein Kinase C (PKC), ERK1/2, and JNK altering protein-protein and protein-RNA binding patterns of this splicing factor (Bomsztyk et al. 2004). Similarly, Sam68 binds elements within pre-mRNA and can become phosphorylated by kinases such as ERK (Matter et al. 2002). An excellent review of these splicing hubs can be found in (Lynch 2007).

Splicing hubs such as Sam68 and hnRNP K are involved in cancer pathology in several ways. First, their expression

levels are often altered. For example, immunostaining of colorectal cancer tissue reveals a significant association between cytoplasmic hnRNP K expression and stage of disease (Hope and Murray 2011). Also, melanoma cell lines and tissues express higher levels of hnRNP K compared to normal melanocytes and benign nevi, respectively (Wen et al. 2010).

Second, aberrant upstream signals typical in cancer can alter normal post-translational modifications and thus localization and activity of splicing factors. Both hnRNP K (Lewis et al. 2000) and Sam68 (Matter et al. 2002) are targets for phosphorylation by ERK—a component of the Ras/MAPK signaling pathway that is frequently hyperactive in cancer due to *BRAF* mutation. For example, in melanoma, enhanced Ras/MAPK signaling through MEK increases the ratio of microphthalmia-associated transcription factor (MITF) MITF(-) to MITF(+) spliced isoforms (Primot et al. 2010). Although implicated as an oncogene in transformed melanocytes, MITF normally regulates melanocyte development (Levy et al. 2006). Citing the pro-proliferative activity of the MITF(-) isoform (Blaustein et al. 2005), Galibert and colleagues propose that up-regulation of MEK in melanoma resulting in altered MITF splicing is a mechanism by which tumor cells achieve enhanced proliferation (Primot et al. 2010). The phosphatase and tensin homolog (PTEN) and Akt pathway is another growth factor signaling cascade frequently mutated in many cancers. Akt is activated by phosphoinositide-3-kinase (PI3K), and phosphorylates several distinct SR proteins such as SRp40 (Patel et al. 2005) and SF2/ASF (White et al. 2010) that ultimately regulate splicing events. For example, aberrant Akt signaling affects splicing of Casp9 in non-small cell lung cancers (Shultz et al. 2010).

Third, alterations in expression and activity of splicing factors hnRNP K and Sam68 can disrupt normal splicing patterns of their targets. For Sam68, such targets have been extensively reviewed and include CD44 involved in cell migration, invasion, and proliferation; Cyclin D1 involved in regulation of the cell cycle; and BCL-X involved in apoptosis (reviewed in Bielli et al. 2011).

Microenvironments affecting translation

Among the groups of proteins that are translationally up-regulated in cancer, growth factors stand out because of their role in proliferation and cancer progression. A deeply integrated growth factor response involving, but not limited to, FGF, Wnt, and β -catenin involves translation (Créancier et al. 2000; Nathan et al. 1997; Pond et al. 2010). Signaling through FGFR results in the phosphorylation of key mediators of the translational response like ribosomal S6. This causes an increase in the recruitment of Wnt, β -catenin, and eIF transcripts to polysomes (Nathan et al. 1997). Phenotypically there is a rapid increase in palpable tumors

in response to FGFR signaling in mice injected with breast cancer cell lines (Nathan et al. 1997).

Several studies have suggested that changes in the translation of FGF contribute to tumorigenicity (Folkman and Hanahan 1991; Vagner et al. 1995). FGF2 can be translated by either IRES or cap-dependant translation. These methods dictate the start site used for translation resulting in different isoforms of FGF2. Normally FGF isoforms show tissue specificity and are required for normal neural development (Créancier et al. 2000; Wagner 1991). The IRES derived isoforms are expressed in transformed cells and under conditions of stress. Given the known role of FGF in cancer, it is not surprising that FGF2 isoforms exhibit tumorigenic phenotypes causing neovascularization of tumor and immortalization of NIH-3T3 cells (Folkman and Hanahan 1991; Vagner et al. 1995). It is becoming clear that perturbations in communication between the FGF pathway and translation contribute to the progression of many tumors (Nathan et al. 1997). FGF is just one example of the interconnectedness of growth factors and translational signaling. Other translationally controlled proteins like ER α , VEGF, platelet-derived growth factor (PDGF), FGF2, IGF-1 receptor, and TGF- β 1 might show similar levels of regulatory complexity (Fraser et al. 2002; Stoneley and Willis 2004).

External stimuli including growth factors, hormones, cytokines, extracellular matrix components, and mitogens activate the PI3K/Akt pathway and affect translation through mTOR (Chung et al. 2002; Hay and Sonenberg 2004; Hennessy et al. 2005; Kroczyńska et al. 2009; Petroulakis et al. 2007; Remy et al. 2004; Stephens et al. 2005). Indeed, several studies show that mTOR regulated by Akt mediates transformation through multiple mechanisms including translational repression (Aoki et al. 2001; Sodhi et al. 2006). Importantly, the RAS/MAPK and PI3K/AKT pathways that both regulate mTOR and translation are among the most frequently altered pathways in cancer. mTOR activity is also regulated by oxygen deprivation and nutrient levels (Holcik and Sonenberg 2005). Convergence of these signals results in an extensive and integrated mechanism that enables cells to respond to the microenvironment.

Hypoxia in the microenvironment

Perhaps one of the most pervasive microenvironmental factors affecting cell phenotype is O₂ availability. Hypoxia occurs when oxygen supply to a tissue is deficient. A state of hypoxia is normal in some contexts such as early embryonic development, but also arises in regions of tumors where vascular networks delivering oxygen have failed to penetrate.

Hypoxia promotes the stabilization of hypoxia inducible factor α (HIF- α) proteins, the best studied of which is HIF-1 α , which dimerizes with HIF-1 β and translocates to the

nucleus to regulate transcription. Cytoplasmic levels of HIF-1 β are stable under hypoxic and normoxic conditions. HIF-1 α protein levels rapidly increase in response to low oxygen resulting from protein stabilization by inactivation of prolyl-hydroxylase which normally targets HIF-1 α for degradation (reviewed in Semenza 2003). Following stabilization, nuclear translocation occurs. In the nucleus, HIF-1 acts as a transcription factor increasing the expression of genes that have hypoxia-response elements (HREs) in their promoter region. HIF-1 activity is associated with genes regulating cell motility and invasion such as fibronectin and matrix metalloproteinase (MMP)-2, ECM-modifying enzymes such as lysyl oxidase (LOX), as well as pluripotency factors including OCT4, NANOG, and SOX2 (Erler et al. 2006; Forristal et al. 2010; Semenza 2003). The hypoxic microenvironment has been implicated in promoting cellular transformation, as well as subsequent tumor development, invasion, and metastatic spread (e.g. Bedogni and Powell 2009).

Splicing response to hypoxia

A few groups have investigated global patterns of AS in response to hypoxia using exon array technology. Using normal human umbilical vein endothelial cells, Zhang and colleagues found 342 exons that displayed differential inclusion in processed transcripts when cells were treated with cobalt chloride to mimic hypoxic stress (Hang et al. 2009). Hypoxic down-regulation of splicing factors heteronuclear ribonucleoprotein (hnRNP) H1 and arginine/serine-rich (SR) 5 in mouse fibroblasts offers mechanistic support to the notion that hypoxia alters global patterns of AS (Greijer et al. 2005).

Hypoxia-associated splicing is also relevant in cancer. A screen of Head and Neck Squamous Cell Carcinoma (HNSCC) samples with either low or high “Hypoxia Scores” (Winter et al. 2007) found several transcripts demonstrating a correlation between Hypoxia Score and the ratio of alternatively spliced transcript isoforms (Moller-Levet et al. 2009). Specifically, this study identified preferred splicing in hypoxia of Laminin α 3 (LAMA3) isoform LAMA3-A, over full length LAMA3-B. While LAMA3 is known to be involved in tumor cell invasion, HNSCC patients in the highest quartile for expression of the LAMA3-A isoform demonstrated decreased survival compared to those with expression in the lower three quartiles. Importantly, this correlation was not seen in patients who expressed the highest relative levels of LAMA3-B. This suggests LAMA3-A is an isoform-specific hypoxia induced prognostic biomarker for disease progression. In breast cancer, AS of CCN1 (Cyr61) is influenced by hypoxia (Hirschfeld et al. 2009). Using RT-PCR and immunocytochemistry, Hirschfeld and colleagues demonstrated that hypoxia promotes a shift in CCN1 splicing toward the aforementioned intron 3-skipping isoform capable of generating full length functional protein.

Cell lines cultured in hypoxia demonstrated increased splicing of the intron-skipping isoform, and stronger cytosolic expression of the corresponding protein. These changes were consistent with increased splicing of the intron-skipping isoform in tumor versus matched normal samples, and increased protein expression in invasive ductal carcinoma cells compared to adjacent non-neoplastic ductal epithelium, with the former presumably representing more hypoxic tissue (Hirschfeld et al. 2009). In neuroblastoma, hypoxic stress mimicked by cobalt chloride influenced the AS of a tumor-suppressing neurotrophin tyrosine kinase receptor (TrkA). Hypoxic stress increased splicing of the TrkAIII isoform normally restricted to neural progenitor cells that was shown to antagonize normal TrkA signaling, thus promoting tumor progression through increased tumor angiogenesis (Tacconelli et al. 2004). From these examples, it is clear that the hypoxic microenvironment can induce splicing of transcript isoforms that promote tumor progression.

Translational response to hypoxia

As the master transcriptional regulator of the hypoxic response, HIF-1 capitalizes on the change from cap-dependant to cap-independent translation in order to accumulate in response to low levels of oxygen (Rofstad et al. 2002). An often overlooked aspect of HIF-1 α regulation is translation. In order to sustain the high nuclear levels of HIF-1 α required for an hypoxic response, mRNA must continue to be translated. It is well established that HIF-1 α contains an IRES that allows for efficient translation during oxygen deprivation and serum starvation (Görlach et al. 2000; Lang et al. 2002). The intranuclear accumulation of HIF-1 requires two types of post-transcriptional regulation: Translation via the IRES and an increase in protein stability act in concert to coordinate extensive changes in protein expression.

HIF-1 α translation can also be affected by tumor promoting driver mutations for example the oncogenic PML deletion as well as KRAS and BRAF mutations increase the translation of HIF-1 α in response to hypoxia (Bernardi et al. 2006; Kikuchi et al. 2009). These studies elegantly demonstrate the combinatorial regulation of tumor progression and suggest how mutation, microenvironmental factors and translational mechanics can have cumulative effects in cancer.

There are many other genes that are up-regulated translationally that contribute to cancer progression. The number of known genes that have IRES sequences is growing rapidly and includes key modulators of pluripotency (OCT4) (Wang et al. 2009) and tumor progression (VEGF) (Huez et al. 1998). Understanding the biological relevance of translating these genes in a cap-independent manner still requires a lot of work, but it offers new and interesting ways of viewing the progression of cancer. Several genes, including SOX2, c-myc, mdm2, uPAR, XIAP, and VEGF have been

shown to be translationally regulated and are important in disease progression (Chung et al. 2002; Ge et al. 2010; Genolet et al. 2011; Graff et al. 2007; Riley et al. 2010; Tan et al. 2000). For example, SOX2 and OCT4 have been identified as being associated with subpopulations of cells with enhanced tumorigenic properties (Ge et al. 2010; Wang et al. 2009). Of the CCN proteins, CCN1 has been shown to be regulated by hypoxia and an IRES (Johannes et al. 1999; Mukudai et al. 2010). Kunz et al. identified CCN1 as a hypoxia-induced gene in melanoma. The absolute expression of CCN1 also correlated with cancer aggressiveness suggesting the expression is being selected for as melanoma becomes more severe (Kunz et al. 2003). Mechanistically, gene expression analysis determined that CCN1 was up-regulated by HIF-1 α and AP-1. Like many other hypoxia regulated proteins, CCN1 has been shown to be capable of cap-independent translation. The over-expression of EIF4F, as is common in many cancers, increases the association of CCN1 mRNA with polysomes (Johannes et al. 1999; Nasr et al. 2012). Under conditions of hypoxia, CCN1 also activates important signalling pathways including MAPK, PI3K, as well as NF- κ B, resulting in the up-regulation HIF-1 α of in gastric cancer (Lin et al. 2008). Interestingly, this up-regulation was shown to be the result of de novo protein synthesis, rather than through stabilization of HIF-1 α as is typically observed. CCN1 affects numerous phenotypes like proliferation, migration and vascularization and offers a compelling mechanism explaining signal integration among the ECM and growth factors (Kunz et al. 2003; Lin et al. 2008). Factors like these are in part responsible for invasive and metastatic cancer phenotypes.

Recently, a study has demonstrated that key pro-tumorigenic proteins are able to evade the hypoxia-induced repression of cap-dependent translation (Uniacke et al. 2012). In this study, it was discovered that hypoxia stimulates the formation of a complex consisting of HIF-2 α , the cap binding protein EIF4E2 and an RNA binding protein (RBM4). This complex is recruited to RNA Hypoxia Responsive Elements (rHREs) in a manner that captures the 5' cap and targets the mRNA for translation. It is not known if this process is utilized to promote the translation of genes such as OCT4 and VEGF; however, it has been shown to regulate pro-mitogenic receptors such as Epidermal Growth Factor Receptor (EGFR) and Platelet Derived Growth Factor Receptor A (PDGFRA).

Regulation of alternative splicing and translation in the progression of cancer

Angiogenesis and VEGF splicing

Angiogenesis is the formation of new blood vessels and is recognized as one of the “hallmarks of cancer” (Hanahan

and Weinberg 2000, 2011) since a rapidly growing tumor necessitates generation of a vascular network to keep tumor tissue supplied with oxygen and nutrients for growth and metabolism. No factor is more integral to the subsistence of tumor angiogenesis than VEGF. Although normally repressed in most adult tissues, VEGF becomes increasingly expressed and promotes an “angiogenic switch” for sustained tumorigenesis in cancers such as melanoma (Ria et al. 2010). Interestingly, AS yields multiple isoforms of VEGF, including a subset of transcripts that actually demonstrate anti-angiogenic activity referred to as VEGF_{xxx}b (reviewed in Ladomery et al. 2007), where xxx denotes the number of amino acids of a particular isoform (Ferrara 1999). The VEGF₁₆₅b isoform prevents normal angiogenesis in response to hypoxia and proliferation in response to VEGF in vitro, and also plays an anti-angiogenic role in vivo. Injection of nude mice with various cancer cell lines over-expressing VEGF₁₆₅b resulted in smaller tumors relative to those in mice injected with VEGF₁₆₅ over-expressing cells. This has been demonstrated for the A375 (Woolard et al. 2004) and Mel57 (Rennel et al. 2008) melanoma cell lines. However, the anti-angiogenic activity of some VEGF_{xxx}b isoforms has recently been disputed. It has been suggested that VEGF₁₂₁b and VEGF₁₆₅b are in fact weakly angiogenic and are up-regulated in some breast cancers relative to normal tissue (Catena et al. 2010). Therefore, there are likely other mechanisms that modulate the angiogenic activity of VEGF_{xxx}b isoforms.

The VEGF_{xxx}b transcripts are the predominant class of isoforms in most normal adult tissues such as renal cortex (Bevan et al. 2008). However, many cancers express the majority of VEGF in the VEGF_{xxx} form. As examples, VEGF_{xxx}b isoforms are endogenously down-regulated in renal cancer compared to normal tissue (Bates et al. 2002), and in metastatic relative to benign prostate tumors (Woolard et al. 2004). In human melanoma, Bates and colleagues used PCR and immunohistochemistry to demonstrate that patients who subsequently developed metastatic disease (within 8 years after samples were taken) displayed higher pan-VEGF to VEGF_{xxx}b ratios during both radial growth phase and vertical growth phase, due to reduced levels of VEGF_{xxx}b isoforms, relative to patients with tumors that did not later metastasize (Pritchard-Jones et al. 2007). These findings suggest that as disease progresses, tumors actively switch splicing in favor of the angiogenic VEGF_{xxx} isoforms, contributing to sustained angiogenesis and tumor progression.

The VEGF_{xxx}b isoforms differ from their VEGF_{xxx} counterparts due only to selection of a more distal splice site in the eighth and most 3' exon. This splice site choice results in six C-terminal amino acids that are divergent in sequence between these two classes of VEGF isoforms and accounts for their drastically opposing functions (Ladomery et al. 2007).

Microenvironmental factors have been shown to promote expression of VEGF. Hypoxia (Liao and Johnson 2007) and

growth factors including TGF- β are obvious candidates to regulate VEGF splicing. However, prior to 2008, only a few factors had been explored in terms of their ability to regulate splicing of VEGF, and no factors had been shown to specifically influence splicing of VEGF_{xxx} transcript isoforms (reviewed in Lodomery et al. 2007). Since then, Bates and colleagues have investigated the impact of TNF- α , IGF, and TGF- β 1 signaling on the VEGF/VEGF_{xxx} splice decision (Nowak et al. 2008). Treatment of two different epithelial cell types with either IGF or TNF- α resulted in increased expression of total VEGF protein and concomitant decreased expression of the VEGF_{xxx} protein isoforms. Specifically, IGF was able to induce a complete splice switch of VEGF in retinal pigmented epithelial cells, from 80 % to a mere 5 % of VEGF spliced as VEGF_{xxx}. Subsequent work demonstrated that IGF-induced splicing of VEGF acts through PKC and SRPK1 (Nowak et al. 2010). Conversely, although TGF- β 1 also increased total VEGF expression, this growth factor skewed splicing toward the anti-angiogenic VEGF_{xxx} isoforms. This change in splicing was shown to be mediated by p38 MAPK signaling (Nowak et al. 2008). The splicing of VEGF is a well-studied example of how the tumor microenvironment can actively perturb AS to favor isoforms that enhance tumor progression. Although VEGF is a major player in the process of angiogenesis, it is likely that changes in the AS of other angiogenic factors likely act in concert with VEGF to collectively promote sustained tumor angiogenesis (Harper and Bates 2008; Lodomery et al. 2007; Nowak et al. 2008; Pritchard-Jones et al. 2007).

Translation of VEGF isoforms in tumor angiogenesis

Despite having a known role in growth and maintenance of multiple tumors, the full regulatory mechanism governing VEGF function has yet to be characterized. Oxygen concentration appears to be one of the most potent regulators of VEGF expression (Stein et al. 1998). The presence of two IRESs in the 5' UTR helps to maintain translation during environmental insult such as hypoxia or other stress conditions and supports VEGF expression (Abcouwer et al. 2002; Stein et al. 1998). The expression of VEGF in response to the microenvironment requires the transduction of multiple signaling pathways that can upregulate translation.

Consistent with the translational regulation of other proteins, the rate of translation of VEGF is affected by PI3K/AKT signaling (Chung et al. 2002; DeFatta et al. 2000; Kevil et al. 1996). For example, the presence of α 6 β 4 integrins was demonstrated to induce VEGF expression via the PI3K/AKT pathway (Chung et al. 2002). In this study, the α 6 β 4-mediated increase in VEGF protein occurred without a concomitant up-regulation of VEGF mRNA. Rather, translation was increased as a result of the

cytoplasmic domain of the β -integrin signaling acting through AKT to decrease the phosphorylation of 4E-BP. It is clear that VEGF regulation is the sum of a number of signals, several of which regulate VEGF at the level of translation (Chung et al. 2002).

From the single VEGF gene, a multitude of alternatively spliced proteins and two alternatively translated isoforms can be produced (Houck et al. 1991; Huez et al. 1998, 2001; Lei et al. 1998; Leung et al. 1989; Poltorak et al. 1997; Tischer et al. 1991). Selection of the upstream start site that dictates the translated isoform is controlled by an IRES. The resulting additional 180 amino acid sequence is highly conserved among mammals suggesting a conserved function (Bornes et al. 2004). Using three expression splice variants with a mutation to prevent further processing and an HA tag for identification, Bornes et al. found that the splice variants showed distinct preferences in start site selection. VEGF₁₈₉ primarily utilized the canonical start codon. VEGF₁₂₁ was expressed exclusively from the upstream codon and VEGF₁₆₅ was expressed equally from both codons (Bornes et al. 2004). Surprisingly, hypoxia had no effect on the pattern of expression of VEGF translational isoforms. It is still unclear what factors regulate this process and what role this extensive regulation plays in cancer. What is becoming clear is that the mechanisms of splicing and translation act cooperatively to determine both expression and protein sequence.

Regulation of epithelial-mesenchymal transition (EMT) in cancer

The epithelial to mesenchymal transition (EMT) is a process fundamental to embryonic development that is sometimes aberrantly induced within tumors. This process involves the loss of epithelial E-cadherin and gained expression of N-cadherin and vimentin, allowing migratory and invasive abilities that ultimately potentiate metastatic spread (Thiery et al. 2009). Along with widespread changes in gene expression, reprogramming of AS (Shapiro et al. 2011; Thiery et al. 2009) and translation coincide with EMT.

Role of alternative splicing in the regulation of EMT

In the tumor microenvironment, basic fibroblast growth factor (bFGF) is an important protein secreted by stromal cells (Villanueva and Herlyn 2008), to which tumor cells are responsive due to expression of receptor tyrosine kinase FGF receptors. FGFR2 is one of these receptors and is subjected to AS. The mutually exclusive exons IIIB and IIIC are included in processed transcripts in normal epithelial and mesenchymal cells, respectively (Yan et al. 1993). Moreover, this transcript undergoes a drastic switch in splicing from including exon IIIB to including exon IIIC during

EMT, with inclusion of exonIIIc associated with reduced affinity of the ligand-binding domain of FGFR2 for FGF7 (Yan et al. 1993). It has also been suggested that both EMT and mesenchymal to epithelial transition (MET) occur within a prostate tumor model, with FGFR2 splicing modulating this potential for bidirectional phenotypic plasticity that enhances malignancy (Oltean et al. 2006).

Ron is a receptor tyrosine kinase for macrophage-stimulating protein that plays a role in cellular invasion through extracellular matrices, an important process in early development that again becomes exploited by some tumor cells during EMT. One of the alternatively spliced Ron isoforms (Δ Ron) lacks exon 11 and translates a constitutively active oncogenic isoform of Ron (Collesi et al. 1996). In both T47D and 293 cells that normally include exon 11 in the majority of Ron transcripts, SF2/ASF over-expression induced increased skipping of exon 11, dependent on an exon splicing enhancer element within constitutive exon 12 (Ghigna et al. 2005). SF2/ASF over-expression in cl.SF2 cells promoted decreased expression of the epithelial marker E-cadherin as assessed by immunofluorescence and RT-PCR, the acquisition of an elongated spindle-shaped morphology, and increased motility in a transwell migration assay—phenotypes common to Δ Ron over-expression and resulting constitutive Ron signaling (Bardella et al. 2004; Collesi et al. 1996; Wang et al. 2004). These data suggest that there is likely a role for SF2/ASF in altering Ron splicing to enhance EMT.

Mena interacts with members of actin remodeling networks important for cytoskeletal changes essential for cell migration. Several cancers express increased levels of Mena (Di Modugno et al. 2004). In breast cancer, Mena expression is positively correlated with an increased capacity of tumor cells for invasion (Di Modugno et al. 2006). Different patterns of AS exist for Mena due to four alternatively included exons. Specifically, the “Mena^{INV}” (Philippart et al. 2008) isoform containing the “+++” exon but lacking the “11a” exon is associated with invasive potential (Goswami et al. 2009). Stable MTLn3 cell lines expressing EGFP-tagged Mena^{INV} and injected into SCID mice produced orthotopic breast tumors with significantly more motile cells, and significantly more lung metastases compared to mice injected with cells expressing either EF3P or EGFP-Mena (Philippart et al. 2008). Mechanistically, Mena^{INV} was shown to contribute to increased invasion and metastasis through regulation of epidermal growth factor signaling and stabilization of invadopodia (Philippart et al. 2008).

Although the functional impact of splice switching events for transcripts such as FGFR2 during EMT remain to be detailed, the regulation of these splicing events has been the focus of comprehensive reviews (David and Manley 2010; Luco et al. 2011). As examples of the above mentioned factors involved in EMT, FGF receptor splicing is regulated

by TGF- β signaling (Shirakihara et al. 2011), while the ERK1/2 target Sam68 affects levels of SF2/ASF involved in EMT and MET splicing regulation as illustrated above in the case of Ron (Valacca et al. 2010). In this vein, it seems likely that an EMT “splicing signature” exists, analogous to the “invasion signature” defined by coordinated transcriptional changes during invasion (Philippart et al. 2008; Wang et al. 2004, 2007). In support of this notion, splicing of FGFR2, Mena, CD44, p120-catenin, and EPB41L5 (all important in EMT) is regulated by the two RNA binding proteins epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2) (Warzecha et al. 2009a, b). An “oncogenic splicing switch” driven by hnRNP H has also been proposed in glioma, promoting splicing of the anti-apoptotic IG20 isoform MADD, and constitutively active Ron (Lefave et al. 2011). Most recently, transcriptome-wide analysis of splicing changes in a Twist-induced model of EMT revealed hundreds of genes with altered splicing patterns, many of which are regulated by three splicing factors ESRP1, RBFOX2, and PTB. Furthermore, a set of nine of these splicing events with validated and robust changes during EMT had predictive value in classifying breast cancer cell lines as either luminal (generally poorly metastatic) or basal (generally more aggressive and metastatic) (Shapiro et al. 2011). The existence of splicing signatures for other processes integral to tumor progression such as angiogenesis has also been hypothesized (Harper and Bates 2008; Ladomery et al. 2007; Nowak et al. 2008; Pritchard-Jones et al. 2007). While this concept might also extend to an overall malignant signature, much remains to be discovered in both regards.

Regulation of translation in EMT

Translation has been shown to control the expression of an important regulator of EMT, Snail. Increased levels of Snail and Y-box factor-1 (YB-1) have been shown in invasive breast epithelial cells (Evdokimova et al. 2009). YB-1 promotes the expression of Snail, N-cadherin, and TWIST. Snail expression is maintained in breast epithelial cells treated with mTOR inhibitors, suggesting that it may be translated via cap-independent mechanisms. Indeed, luciferase activity detected from a Snail-5'UTR-luciferase reporter suggests that its translation is maintained during mTOR inhibition (Evdokimova et al. 2009). Mechanistically it was determined that YB-1, when phosphorylated by AKT, is a repressor of cap-dependent translation which allows for the up-regulation of IRES-containing mRNAs including Snail (Evdokimova et al. 2006). Furthermore, other studies have shown that HIF-1 α and Snail may be translationally regulated simultaneously to support the down-regulation of E-cadherin associated with EMT (Imai et al. 2003). These data suggest that EMT is, in part, regulated by hypoxia and YB-1 through IRES-dependent regulation of Snail.

Some of the most prominent communication between splicing and translation has been described in the context of EMT. The translation of zinc finger E-box binding homeobox 2 (ZEB2) mRNA during EMT is regulated by a novel mechanism in human colorectal cancer. In one study, EMT was induced by transfection of Snail (Beltran et al. 2008). The amount of ZEB2 protein was increased during EMT without the expected transcriptional activation of the gene. It was determined that a previously discovered natural antisense transcript (NAT) binds to the 5'UTR of the ZEB2 transcript, preventing splicing and leading to the retention of the IRES. The regulation of ZEB2 offers a new and exciting model of regulation for EMT wherein translation and splicing work in combination.

Recent data suggest that splicing and translation may co-activate the same pathways to promote similar phenotypes. hnRNP E1 is a translational regulator as well as a regulator of splicing. In a review by Chaudhury et al., the multiple roles of hnRNP E1 as a translational regulator and as a splicing factor are well summarized (Chaudhury et al. 2010a). hnRNP is integral in TGF- β -mediated EMT. Using a number of models including oncogenic RAS-

transformed mouse mammary epithelial cells, Howe and colleagues showed that TGF- β upregulates translation of Dab2 and ILEI, contributing to EMT (Chaudhury et al. 2010b). Both Dab2 and ILEI contained TGF- β -activated translation (BAT) elements in their 3'UTR. BAT elements are bound by the BAT-binding messenger ribonucleoprotein (mRNP) complex that silences translation. One component of this complex, hnRNP E1, is phosphorylated in response to TGF- β signaling via AKT2 activity, disrupting binding of Dab2 and ILEI and increasing the efficiency of translation. Though the full extent of interaction between splicing and translation has yet to be discovered, research to date indicates convergent effects on phenotype, co-operative regulation of single genes and even shared components of the machinery required for both types of regulation.

Conclusion

Cancer is characterized by cellular adaptation to changing microenvironments and by hallmarks such as angiogenesis and EMT. These critical processes are mediated by the

Fig. 3 A summary of how microenvironmental factors typically affect signaling pathways regulating alternative splicing and translation. Blue arrows indicate activating effects, although not necessarily an active protein, and red bars indicate inhibiting effects. Extracellular activation of PI3K causes an activation of AKT resulting in the phosphorylation of SR proteins and mTOR, directly altering splicing patterns and the dynamics of translation, respectively. Inhibition of mTOR enhances

cap-independent translation through derepression of 4EBP causing inactivation of eIF4E. The RAS/MEK pathway is also involved in promoting cap-independent translation through other eIF proteins. Components of both of these pathways (AKT and ERK), also phosphorylate SR proteins, altering their RNA binding specificities and thus patterns of splicing

increased transcription of a myriad of genes, including VEGF, FGFR, and Snail. In addition to these classical changes in gene expression, it is clear that alterations in the splicing and translation of these genes also contribute to rapid expression of pro-tumorigenic proteins. Furthermore, changes in transcription, splicing, and translation do not take place in isolation, as there is much evidence for coordinated regulation of these processes. First, AS actually takes place co-transcriptionally. According to the kinetic model, a slower rate of transcription allows inclusion of weak alternative exons, while the recruitment model posits that specific splicing factors can bind RNA polymerase II to increase their local concentration at target splice sites and thereby strengthen the interaction (Kornblihtt 2007; Luco et al. 2011). Second, enzymes that modify histones to modulate the accessibility of specific genetic loci to the transcriptional machinery can also modulate AS events through binding small nuclear ribonucleoproteins components of the spliceosome (reviewed in Luco et al. 2011). For example, EMT-associated decreases in FGFR2 histone 3 lysine 27 trimethylation (H3K27me3) and H3K4me3, and increases in H3K36me3 and H3K4me1 was shown to causally promote the corresponding FGFR2 splice switch discussed above (Luco et al. 2010).

Along with several other aspects of mRNA metabolism, splicing and translation are both regulated by the SR proteins (Huang and Steitz 2005). Srebrow and colleagues used the fibronectin gene to illustrate that the PI3K/AKT pathway can influence SR protein activity and thus regulate both AS and translation in an isoform-specific fashion (Blaustein et al. 2005). The splicing factor SF2/ASF also recruits mTOR to some transcripts (Michlewski et al. 2008), while hnRNP K is involved in regulation of translation as previously illustrated. Collectively, these examples emphasize the widespread perturbation of post-transcriptional regulation in cancer.

Thus, we propose an integrated model for changes in gene expression during cancer progression (Fig. 3). That is, tumor cells respond to and forge their microenvironment via modulation of gene expression through not only classical transcriptional responses, but also inter-dependent post-transcriptional processes including AS and translation. These changes favor tumor cell plasticity, enabling pro-tumorigenic processes affording cellular growth and metastasis. Future work that considers post-transcriptional levels of regulation will do much to identify novel genes and mechanisms that contribute to tumor progression.

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