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REVIEW

Transcription factors: their potential as targets for an individualized therapeutic approach to cancer

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Pro-cancer signals are controlled by the expression and transcription of oncogenes. Transcription of DNA is dependent on the spatially and temporally coordinated interaction between transcriptional machinery (RNA polymerase II, transcription factors (TFs)) and transcriptional regulatory components (promoter elements, enhancers, silencers and locus control regions). Unique TFs have been identified in association with cancer. This review summarizes key oncogene-related TFs and organizes their pro-cancer activity according to the six hallmark functions (self sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis and metastatic spread) proposed as constituting the infrastructure of the malignant process.
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Introduction

Recent developments in molecular biology and biomolecular technology show promise for dramatically altering current strategies of cancer treatment. Each cancer is a co-opted complex adaptive network of dynamically evolving spatial–temporal biomolecular interactions. Multilevel functional complementation results from the interactions between spatial, temporal and/or process-dependent modules at each organizational level (genome → transcriptosome → proteome → metabolome) that crossreact producing a functional organizational hierarchy.[1] Work is now underway to integrate theoretical and experimental programs so as to map out and model, in qualitative and quantifiable terms, topological and dynamic properties of networks that control the behavior of cancer (cells). Development of high-throughput data collection techniques on both the genomic (for example, DNA and oligonucleotide microarray) and the proteomic (for example, LC/MS) level allows for simultaneous interrogation of the status of a cell's components at any given time and is contributing to our knowledge and understanding of the organization and, at a slower pace, the mechanics of cancer co-expression networks. Likewise, the emergence of GeneChip Mapping arrays (Affymetrix Inc., Santa Clara, CA) and ChIP-on-chip analysis[2] has produced a rapid expansion in our understanding of both the extent and phenotypic effects of transcription promoter polymorphisms.[3,4] In one study, 50% of promoter sequence variants altering gene expression more than 1.5-fold were located within 100 bases 5′ to the transcription start site.[4] Most regulation appears to occur at the level of transcription initiation, prompting attempts to establish topologies of transcriptional regulatory networks to allow for the analysis of regulatory links (transcription factor (TF) → gene) in addition to expression and co-expression changes of specific genes[5,6] to delineate tumor versus normal tissue differences in a dynamic processing (rather than static) mode.

Transcription initiation is dependent on the spatially and temporally coordinated interaction between the transcriptional machinery (RNA polymerase II, general TFs, activators and co-activators) and the transcriptional regulatory element, which includes the docking-site core promoter, proximal promoter elements (usually near a CpG island) and distal promoter elements, enhancers that are functionally similar to proximal promoter elements, silencers (the binding sites for repressors), insulators that minimize the effect of neighboring genes and locus control regions, which regulate gene clusters.[7,8] Dysregulation of transcriptional and consequent post-transcriptional processes contributing to both cancer initiation and persistence can occur by aberrant activation, repression and/or temporal/spatial dyscoordination as well as by structural changes including mutations (*BRG1* and *BRM*), translocations (*cMYC* in Burkitt's lymphoma) and fusion (*BCR-ABL*).[8]

Hanahan and Weinberg[9] have identified six hallmark functions that provide the necessary infrastructure for the malignant process. These include (1) self-sufficiency in growth signals, (2) insensitivity to growth-inhibitory signals (antigrowth), (3) evasion of programmed cell death (apoptosis), (4) limitless replicative potential, (5) sustained angiogenesis and (6) tissue invasion and metastatic spread. This paper will provide a focused

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review of those TFs that, based on their associated signal transduction pathways, have been implicated in the carcinogenic process and, as such, have potential as therapeutic targets.

TF networks

To prepare this review, a list of 320 somatic oncogenes compiled by the Sanger Institute (http://www.sanger.ac.uk/genetics/CGP/Census/) was searched. The list was cross-referenced with the database at the National Center for Biotechnology Institute (http://www.ncbi.nlm.nih.gov/) to find unique TFs that are linked to cancer. Of the 63 original TFs identified, 19 have been culled that uniquely or differentially modulate gene expression in cancer with a potential for therapeutic manipulation. We also attempt to contextualize the TFs by classifying them on the basis of their hallmark functional activity as defined by Hanahan and Weinberg (Table 1).

Self-sufficiency in growth signals

Unlike normal cells, cancer cells have the capacity for autochonous growth, thus providing an unregulated mechanism of proliferative stimulation free of the constraints imposed within normal tissues.[9]

NF-κB

The TF nuclear factor (NF)-κB is a family of five reticulendotheliosis (REL) proteins that can be broken into two groups: group 1 contains RELA (p65), c-REL and RELB and group II, NF-κB1 (p105) and NF-κB2 (p100).[10] The NF-κB protein influences gene transcription through a series of events that allows it to translocate to the nucleus. Inhibitor of NF-κB (IκBα) binds to NF-κB in the resting state, thereby sequestering the complex in the cytoplasm in an inactive conformation. Activation of the TF proceeds through a cascade of events consisting of the IκB kinase-mediated phosphorylation of IκBα, ubiquitination and consequent proteosomal degradation resulting in nuclear translocation of NF-κB.[11] NF-κB is constitutively active in several cancer types and has been associated with the regulation of cell proliferation, cell survival, invasion, metastasis and inhibition of apoptosis through the inhibitor of apoptosis (IAP) constellation and GADD45.[10]

Inhibition of NF-κB signaling has been shown to retard tumor formation.[12] NF-κB downregulation inhibits the expression of vascular endothelial growth factor (VEGF) with a consequent reduction in vascular formation[12] and interacts with other TFs.[13,14]

AP-1

The activator protein-1, AP-1, dimer is comprised of c-Jun, JunB, JunD, Fos, FRA-1 and FRA-2. The dimer combinations, as well as their specificity and stability, are determined by the composition of its leucine zipper region. Malignant cell proliferation is regulated by c-Jun through the repression of tumor suppressor genes, as well as through the induction of cyclin D1 transcription,[15] whereas JunB and JunD are more frequently negative regulators. c-Jun is unique in binding directly to a variant AP-1 site in the p53 promoter PF-1 site, characterized by a single base-pair exchange, to negatively regulate p53 transcription.[16] Notably, AP-1 inhibition has been shown

Table 1 Hallmark ascription[9] of oncogenic transcription factors

Transcription factor	*Hallmark traits*					
	Self-sufficiency in growth signals	*Insensitivity to growth-inhibitory signal*	*Evasion of programmed cell death*	*Limitless replicative potential*	*Sustained angiogenesis*	*Tissue invasion and metastasis*
AP-1	X		X		X	X
AR	X					
ATF-1						X
BRN-3b		X				
C/EBPα		X				
CREB			X		X	
E2F-1				X		
ETS-1					X	X
EWS/ETS				X		
FOX03a			X			
HIF-1α/HIF-1β(ARNT)					X	X
Myc	X				X	X
NF-κB	X		X		X	X
PEA3						X
RARα	X					
RB1				X		
SP-1					X	
STAT3		X	X		X	
STAT5		X	X			
TP53			X			

to stymie breast cancer cell proliferation by suppressing mitogenic signals from peptide growth factors (insulin-like growth factor-1, epidermal growth factor, basic fibroblast growth factor and β-heregulin).[17]

The different AP-1 subunits have different pro-cancer effects. In general, as noted, Jun and Fos proteins are associated with proliferation and inhibition of apoptosis, Fra-1 proteins enhance angiogenesis, and JunB and JunD can either stimulate or inhibit proliferation (generally, the latter). Modulation of AP-1 activity may be a novel approach to reducing malignant transformation; however, targeting to the malignant (as opposed to non-malignant) tissue will be necessary[18] as AP-1 possesses both oncogenic and antioncogenic characteristics.

Steroid receptors

Cell proliferation in prostate cancer can be attributed to androgen receptor TF, which binds to its cognate response element in DNA.[19] Although chemical androgen ablation is associated with clinical response in over 80% of men with advanced prostate cancer,[20] the disease inevitably becomes hormone refractory and unresponsive to androgen suppression. In some tumor models, androgen receptors are either mutated or amplified resulting in enhanced ligand occupancy or activation by cross-talk with other growth factors. Disease progression ensues with eventually fatal results.[19,20]

Retinoic acid receptors (RARs) are members of a group of ligand-dependent TFs that also include steroids, thyroid hormone and vitamin D receptors.[21] RARs have several functions; however, the transcriptional activation of retinoic acid and DNA binding plays an important role in gene expression.[22] Experiments using human SCC71 squamous cell carcinoma lines showed that the cell proliferation was differentially dose-dependent on retinoic acid regulation. Low doses of retinoic acid increased proliferation of SCC71 by epidermal growth factor activation of ERK1, resulting in an increased expression of S- and G2-phase cyclins and cyclin-dependent kinases, increased Rb phosphorylation and increased E2F-1 DNA-binding activity. On the other hand, higher doses produced inhibition of ERK1 expression.[22] In other studies, increased expression of RARα is associated with cellular proliferation.[23] In an analysis involving advanced ovarian cancer patients conducted by Kaiser *et al.*,[24] elevated expression of RARα was shown to be a poor predictor of survival. Elevated expression was also associated with poor prognosis in patients with oral squamous cell carcinoma, prostate and breast cancer.[23,25,26]

Insensitivity to growth-inhibitory signals

The cell cycle can be temporarily held in a quiescent state (G0), until activation of the appropriate re-entry signal. Alternatively, in mature differentiating cells, proliferation is permanently halted by entry into the post-mitotic state. In malignancy, dysregulated TFs circumvent cell cycle checks and balances, thereby creating insensitivity to normal growth-modulating signals.[9]

Myc

Recent estimates suggest that c-Myc transcription regulates up to 15% of genes across different species. The myc TF family, which includes c-Myc, N-Myc and L-Myc, interacts through formation of heterodimeric complexes with proteins MAX and MAD as transcription regulators.[27] During cellular differentiation, there is a shift in binding from c-Myc/Max to Mad/Max; the latter competing for binding sites with the c-Myc/Max heterodimers.[28] Overexpression of c-Myc within the complex is associated with promoting cell cycle progression and inhibition of cell differentiation.[28,29]

N-Myc overexpression has been identified in several types of cancer (retinoblastoma,[30] small-cell lung cancer[31] and medulloblastoma[29]) and has been shown to affect angiogenesis, in part, through the modulation of IL6.[32] Hatzi *et al.*[32] have recently demonstrated that N-Myc amplification enhances the malignant phenotype in neuroblastoma.

Brn-3b

Transcription factor Brn-3b belongs to the class IV POU domain family normally expressed in neurologic and reproductive tract tissues. Overexpression of Brn-3b causes increases in growth rate and proliferation.[33] Dennis *et al.*[33] suggest a role for Brn-3b in regulation of mammary cell growth. Non-malignant mammary cells or benign tumors do not express significant levels of Brn-3b. Budhram-Mahadeo *et al.*[34] found that increased mRNA and protein levels of Brn-3b repress BRCA-1 promoter activity and downregulate BRCA-1 expression. Modification of Brn-3b TF expression in human breast cancer cells correlates with heat shock protein-27 expression.[35] Heat shock protein-27 has been associated with growth, invasiveness and resistance to chemotherapeutic drugs.[35]

Evasion of programmed cell death

Programmed cell death, or apoptosis, constitutes one of the major mechanisms of cellular attrition. Apoptotic signaling is multifactorial and multiplex with both intrinsic and extrinsic components. Tumor development, growth and maintenance are critically related to the control of apoptotic function.[9]

p53

The p53 protein is made up of 393 amino acids that can be divided into four functional and structural domains. There is an acidic amino-terminal domain, a core DNA-binding domain, a tetramerization domain and a C-terminal regulatory domain. The N terminus positively regulates gene expression.[36] The p53 protein balances synthesis and repair, cell cycle arrest, senescence and apoptosis. Mutations of p53 occur in approximately 50% of all cancers and are generally associated with a worse prognosis as well as a higher 'resistance to treatment'.[37]

Under normal conditions, p53 is activated by post-translational modifications, such as acetylation and phosphorylation. This stabilizes and activates p53 in response to DNA damage resulting in either cell cycle arrest or apoptosis.[38] The exact response of p53 to DNA damage may be regulated by p300 in a model proposed by Iyer *et al*.[38] Low levels of p300 in colorectal cancer cells resulted in increased apoptosis in part because of increased p53 stability, which leads to reduced p21 transcription and increased PUMA activation. However, when p300 was not limiting, the proapoptotic pathway expressed minimal activation. The transient activation of p53 resulted in the transactivation of p21, which promoted cell cycle arrest. Several preclinical and clinical trials have now been performed validating the hypothesis that replacement of wild-type p53 function through gene replacement would promote anticancer activity and enhanced apoptotic activity.[39,40]

STAT

Signal transducer and activator of transcription (STAT) is a family of seven proteins, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. They are latent cytoplasmic TFs that are activated by cytokines, growth factor receptors and peptides.[18] These extracellular signals associate with and activate JAK (Janus kinases), which, in turn, catalyze the tyrosine phosphorylation of the receptor-bound STAT, which, following dimerization, translocates to the nucleus to regulate gene expression. With regard to their role in malignancy, STAT3 and STAT5 have been shown to promote cell proliferation and prevent apoptosis in different cell types.[41] STAT3 activates IL-6-responsive genes[42] and is constitutively activated in IL-6-dependent multiple myeloma cell lines as well as in bone marrow mononuclear cells from multiple myeloma patients. Furthermore, it has been shown that apoptotic resistance is a result of constitutive activation in hematological malignancies, inhibiting STAT3-promoted apoptosis in leukemic large granular lymphocytes.[43] Niu *et al*.[44] have also demonstrated a mechanism whereby STAT3 inhibits p53 gene transcription. Blocking STAT3 in this scenario could provide a novel approach to re-establishing p53 expression in dysregulated cells.

STAT3 also mediates angiogenesis following epidermal growth factor receptor activation and STAT3-Src kinase, which in turn induce VEGF. Nui *et al*.[45] also showed a correlation between VEGF expression and STAT3 activity in several human cancer cell lines. In a study of structure–activity relationships of five cucurbitacin (Cuc) analogs, Sun *et al*.[46] have deconstructed the JAK/STAT3 relationship. A structure–activity relationship of Cuc Q demonstrated the ability to inhibit STAT3 and induce apoptosis, but not inhibit JAK2, Scr, AKT, ERK or JNK in nude mice xenografts. However, myeloma, breast cancer and prostate cell survival could be curtailed by targeting the JAK kinase family to block STAT3 activation.[47] Apoptosis of breast cancer cells could also be induced by blocking STAT3 with Src kinase inhibitors.[47]

STATS 1 and 5 are activated in *BCR-ABL* fusion-positive CML patients. Constitutively activated STAT5 is also necessary for *BCR-ABL*-induced transformation. BCR-ABL tyrosine kinase inhibitors can block STAT5 signaling[48] with consequent growth arrest and induction of apoptosis. Tommi *et al*.[49] showed that STAT5 is activated in human prostate cancer cells. Inhibiting STAT5 leads to prostate cancer cell apoptosis.

FOX03a

FOX03a, formerly known as FKHRL-1, is a member of the Forkhead box protein group, the downregulation of which is associated with cell survival. Under normal conditions, following dephosphorylation, FOX03a translocates into the nucleus and triggers apoptosis through target genes (that is, Fas ligand gene).[50] AKT can inhibit the translocation of FOX03a to the nucleus through phosphorylation, thus inhibiting apoptosis and thereby enhancing cell survival.[51] FOX03a is a key regulator of estrogen receptor (ER)α gene transcription and has been linked to the expression of ERα in breast cancer cell lines.[50] Treatments with tamoxifen and estrogen withdrawal induce cell cycle arrest, which can be explained through the interaction of estrogen, ERα and Forkhead TFs.[52]

Limitless replicative potential

Eukaryotic cell replication results in the progressive shortening of the chromosomal telomere region. With the exception of stem cells and germ cells, normal cells lack telomerase resulting in a physical limit to the number of times a cell can undergo mitosis (generally 60–70 doublings: the Hayflick effect).[14] Telomerase is a reverse transcriptase that elongates the telomere region by adding a 'TTAGGG' repeat sequence at the 3′-end.[53] Tumor cells acquire the ability to produce and upregulate telomerase, thus achieving immortality.[9]

EWS/ETS

Ewing's sarcoma is characterized by the expression of a t(11;22)(q24;q12) translocation-induced EWS/ETS TF family member, most commonly EWS-FLI1.[54] This is a novel TF that targets telomerase. In cooperation with cyclic AMP-responsive element-binding (CREB) protein, EWS/ETS has been connected with the TERT, telomerase reverse transcriptase, and the transcription initiation complex. siRNA knockdown of EWS/FLI1 in Ewing's sarcoma cells reduces telomerase activity and TERT mRNA expression.[55] Furthermore, the same group confirmed telomerase stimulation by *EWS/ETS* fusion proteins in Ewing's sarcoma tumors by upregulation of TERT gene expression.

E2F

The TF E2F, also affected by RAR, is regulated through the phosphorylation of retinoblastoma susceptibility gene (RB1). The phosphorylation and dephosphorylation of pRB in late G_1 initiate the expression of genes necessary

for the transition to the S phase of the cell cycle. pRB phosphorylation is primarily regulated by the cyclin D/CDK 4/6 complex and their interaction with the CDK inhibitor p16. The loss of RB1 or pRB expression is found in many tumors[56] including retinoblastoma,[57] bladder carcinoma[58] and malignant neuroendocrine lung carcinoma.[59] E2F-1 dysregulation has been associated with gastrointestinal and lymphatic tumors.

Sustained angiogenesis

Angiogenesis is a highly regulated process by which new blood vessels are, albeit dysfunctional, formed or co-opted.[9] Tumor development and metastatic spread depend on an active angiogenic process to establish a supply of oxygen and nutrients, as well as a pathway for waste removal.[60] Hypoxia is a stress that modifies biological processes such as cell proliferation, angiogenesis, metabolism, apoptosis and immortalization, all of which are necessary for cancer cell viability.[61]

Hypoxia-inducible factor-1α

Hypoxia-inducible factor-1α responds to oxygen depletion in the cellular environment by forming a heterodimer with a second TF, aryl hydrocarbon receptor nuclear translocator, the hypoxia-inducible factor-1β subunit. Hypoxia-inducible factor-1α is thereby stabilized and gene transcription ensues through hypoxia response elements. This leads to the upregulation of several tumor progression-associated genes, one of which is VEGF, a known key angiogenic factor.[62] A study involving 92 ER-negative breast cancer patients suggested a relationship of aryl hydrocarbon receptor nuclear translocator splice variation with drug resistance and angiogenic induction.[62]

CREB

Multiple external stimuli as well as an intrinsic oxygen-sensing mechanism within the cell converge to activate CREB protein, a TF that promotes angiogenesis and resistance to apoptosis.[61] The hypoxia-response genes include the angiogenic gene VEGF[63] and the proapoptotic genes IAP2[64] and Bcl-2.[65] VEGF phosphorylates CREB at serine 133, which leads to DNA binding and transactivation.[63] The IAP2 gene promoter utilizes CREB-binding element to enhance activity under severe hypoxic conditions.[64] Using HCC transfected with CREB300/310, in which the two cysteine residues at positions 300 and 310 were converted to serine, resulting in enhanced CREB-binding efficacy and mediated gene expression, implanted mice responded with dramatically enhanced tumor growth.[61] When serine 133 was replaced with alanine (CREB300/310/133) producing a dominant negative, growth of implanted tumor cells was inhibited.

CREB along with activating transcription factor (ATF)-1 is upregulated in metastatic melanoma cells; overexpression gives rise to the metastatic phenotype through two mechanisms. CREB/ATF-1 can regulate the metalloproteinase MMP-2 and adhesion molecule MCAM/MUC18 gene to promote invasion. Using an inhibitory anti-ATF-1 single-chain antibody fragment (ScFv) to inhibit the transcriptional activity of ATF-1, Jean and Bar-Eli[66] showed that CREB was significantly reduced in melanoma cells and that subcutaneously transplanted tumors in nude mice underwent massive apoptosis in response to ScFv anti-ATF-1.

SP-1

SP-1, a member of the Sp Krüppel-like family, is a ubiquitously expressed TF that controls many genes. Some genes have one promoter-binding site, whereas others have many. A single SP-1-binding site in a promoter can be viewed as a 'on/off' switch for gene expression. However, genes with multiple SP-1 promoter sites, such as oncogenes, are more likely to show modulated gene expression. Human pancreatic cancer cell lines and cancer tissue,[67] breast cancer cell lines and cancer tissue,[68] gastric carcinoma,[69] and thyroid carcinoma[70] have all shown overexpression or a higher binding activity of SP-1, which correlates with the upregulation of VEGF,[67] urokinase plasminogen activator and urokinase plasminogen activator receptor,[68] and epithelial growth factor receptor and epidermal growth factor receptor.[69,71] Wei *et al*.[72] explored the use of COX-2 inhibition to reduce Sp1 DNA-binding and transcriptional activity. Suppression of Sp1 activity appeared to reduce VEGF expression in nude mice suppressing angiogenesis, tumor growth and metastatic spread.[72]

Tissue invasion and metastasis

Metastatic spread involves the migration of cancer cells from one place to another through the lymphatic system or bloodstream. This is the ultimate cause of death in 90% of patients with advanced cancer.[73] Transcriptional control of the metastatic process is complicated and involves several TFs including many of the previously mentioned TFs as well as the ETS family of TFs.

ETS

The ETS family has the ability to activate the transcription of matrix proteases urokinase-type plasminogen activator, collagenase I (MMP-12), stromelysin I (MMP-3) and gelatinase B (MMP-9).[74] Their dysregulated expression is associated with the metastatic potential of tumor cells.[75] The ETS family is divided into subfamilies based on the location of the 84 amino-acid sequence, or ETS domain, that binds to the core DNA-binding motif C/A GCA A/T.[76] It is most likely to promote the development of malignant phenotypes through translocation or deletion, as in Ewing's sarcoma and chronic myeloid leukemia. However, polyoma enhancer activator 3 (PEA3) (also known as E1AF and ETV4) and ETS-1 also play a role in tumor progression through metastatic potentiation and angiogenesis.[76]

PEA3

Although normal human cells show a weak expression of PEA3, PEA3 is overexpressed in several cancer types including breast[77,78] and ovary.[78] Increased expression of PEA3 is associated with the overexpression of HER2/Neu in clinical breast cancer specimens.[77] Benz *et al*.[77] found neu-induced tumors to have differentially high levels of PEA3 mRNA in comparison with the surrounding mammary epithelium. Mammary tumor metastases to the lungs exhibit overexpression of PEA3, which is also indicative of overall worse survival. Co-expression of PEA3 and HER2 are associated with a significant increased rate of recurrence in women with breast cancer compared with HER2 overexpression alone.[79] Shephard *et al*.,[80] using transgenic mouse models, showed that the downregulation of PEA3 could be a therapeutic target in the regulation of HER2/Neu and ultimately breast cancer.

Nitrous oxide (NO) plays a role in carcinogenesis, invasion and metastatic spread of colorectal cancer cells. NO affects β-catenin, which regulates PEA3, which activates the COX-2 gene. COX-2 overexpression expression is related to colorectal cancer and disease progression. Liu *et al*.[81] propose a sequential pathway for COX-2 activation: NO→β-catenin TCF/LEF→PEA3→COX-2. The inhibition of COX-2 also suppresses SP-1, reducing angiogenesis by suppressing VEGF. Inhibiting this pathway through the downregulation of PEA3 may provide a novel therapeutic approach here as well. ETS-1 also regulates gene expression associated with metastatic potential by inducing angiogenic growth factors in endothelial cells.[76,82] ETS-responsive elements are present in the promoter region of VEGF, which may explain a statistical correlation between ETS-1 and VEGF expression data in cancer cells.[74] Iwasaka *et al*.[82] performed a series of *in vivo* experiments using human umbilical vein endothelial cells, which showed that ETS-1 plays a role in angiogenesis, regulating proteases and the migration of endothelial cells.

Metastatic spread is directly linked to the glycolytic bonding of β1→6 Asn-linked oligosaccharides.[83] An antisense oligonucleotide for ETS-1 remodeled endothelial cell properties to inhibit the cell from migrating through basement membranes.[84] Lung metastases of mouse melanoma cells expressing high levels of *N*-acetylglucosaminyltransferase-V were suppressed by transfection of *N*-acetylglucosaminyltransferase-III, which replaces a β1→6 glycolytic bond with a β1→4 bond, further demonstrating the importance of oligosaccharides to metastatic spread.[85] ETS-1 shows pathophysiological significance with regard to malignancy potential. Its increased expression can be correlated with increased expression of *N*-acetylglucosaminyltransferase-V.[86]

Therapeutic vision

In silico modeling has revitalized and expanded our understanding of the carcinogenic process (initiation, promotion and maintenance) as an integrated, dynamic and robust multilevel hierarchical network. Malignancy is an evolved co-opted 'system' maintaining the robust characteristics of its ancestor normal tissue. The qualitative and quantitative dysregulation of 'normal tissue' molecular mechanisms resulting in the malignant process either establishes or supports its functional infrastructure that includes (1) self-sufficiency in growth signals, (2) insensitivity to growth-inhibitory signals (antigrowth), (3) evasion of programmed cell death (apoptosis), (4) limitless replicative potential, (5) sustained angiogenesis and (6) tissue invasion and metastatic spread.[9,67] The dysregulation of transcriptional regulatory structure and/or mechanisms is clearly an integral component in this process as shown by the number of TFs encoded by oncogenes and tumor suppressor genes (Table 1). As illustrated in Figure 1, the potential regulatory steps for the expression of protein-coding genes amenable to therapeutic targeting form a complex network rather than a sequenced pathway from transcription initiation and elongation through mRNA processing, transport, translation and post-translational modifications.[87,88] A key functional hub in this network appears to be located at the level of transcription initiation.[8] With exponentially accruing data from patient-derived fresh tissue with less dependence on xenograft, transgenic and cell-line-derived specimens and more sophisticated modeling approaches to analyze, cull, correlate and integrate static and dynamic data sets,[89] the potential for gene-based therapies capable of targeting key components of the transcription complex has raised expectations that, one day, we will be able to target individual patient tumor-specific and cancer-dependent components.[90]

One approach being used at the Mary Crowley Cancer Research Centers employs individual patient-derived tumor and normal comparator tissue specimens for 2D-differential in-gel electrophoresis to determine differential tumor/normal tissue protein expression distributions followed by functional annotation and confirmation of mRNA overexpression (Affymetrix). Using current databases but prioritizing patient-derived databanks, 'cancer

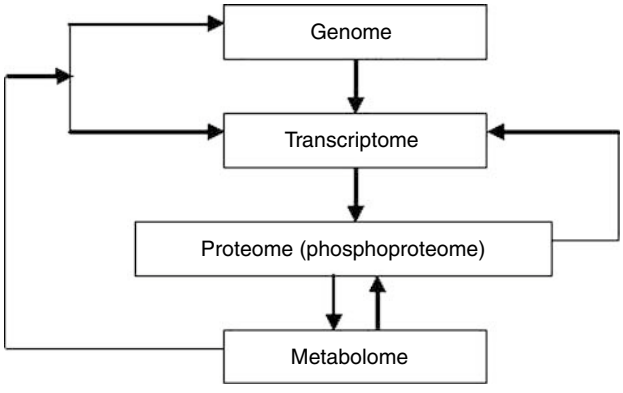

Figure 1 The genome contains all the coded and non-coded information of the organism. Transcriptosomes switch on the specific genes coded through transcription to produce a proteome. The metabolome can be used to classify the proteome's phenotype, which influences subsequent gene expression through transcription.

gene' likelihood and degree of connectivity are assessed. shRNA silencing is validated and consequent proteomic–genomic changes ascertained. These consequent changes are biologically contextualized and sequentially silenced with the goal of reaching a critical functional threshold, the 'debacle point', resulting in system failure (or network malfunction). Optimized targeting strategies should include both inhibition and activation of gene expression. The latter is now feasible with recent developments in zinc finger protein engineering[91] and activating RNAs.[92] Table 1 lists TFs encoded by oncogenes and suppressor genes that we believe the literature supports as reasonable initial targets. Table 2 summarizes the preclinical data involving animal models in which these putative targets are explored.

Targeting TFs as a therapeutic strategy in cancer patients raises three major concerns: selectivity, specificity and differential sensitivity. The global inhibition of a specific TF is likely to result in serious side effects.[90] This requirement for selective targeting necessitates optimization through cancer-directed delivery and cancer-specific effector activation (that is, using cancer-specific promoters, such as hTERT).[20]

Transcription factors in the same family also have similar or shared motifs, or structures, but different functions (for example, STAT1 versus STAT3, c-jun versus jun B). This presents the second challenge. For treatments to be effective, the targeting moieties will have to be able to distinguish between TFs of the same family. Finally, quantitative rather than qualitative differences between cancer and normal tissues reflecting disruptions and divergences in temporal patterns and concentration gradients require attention to differential sensitivity. With the resurgence of systems biology applications, process dynamics, in addition to static analytics, is beginning to be addressed and applied in oncology.[89]

The goal of an effective individualized therapeutic approach to cancer is beginning to come within our sight. Many factual, technical and conceptual hurdles remain and, as they are successfully passed, others seem to appear. Yet, progress is undeniable. TFs, the transcriptional machinery and the transcriptional regulatory element are novel targets for therapeutic attack. Our increased knowledge of transcription mechanics and the transcriptional regulatory network has led to a better understanding of the critical role these play in cancer. Recent developments in delivery vehicles and directed targeting (for example, liposomes, nanoparticles and aptamers) along with more accurate and potent effectors (that is, shRNA, siRNA, ribozymes, antisense oligonucleotides, small-molecule inhibitors and zinc finger proteins) are poised for translation into the clinic.

Table 2 Preclinical justification for using transcription factors

Transcription factor	*Justification summary*	*Reference*
AP-1 (c-Jun)	Inhibition-reduced breast cancer cell proliferation in mice	Liu *et al*.[17]
AR	Downregulation resulted in prostate tumor size reduction in mouse xenograft	Eder *et al*.[93]
ATF-1/CREB	Knockdown in mice resulted in subcutaneously transplanted tumor-size reduction through apoptosis	Jean[66]
BRN-3b (POU)	Results suggest that Brn-3b elevation in breast cancer is a significant transcription factor in regulating mammary cell growth	Dennis *et al*.[33]
C/EBPa	C/EBPa knockout mice showed abnormal lung cancer cell proliferation	Halmos *et al*.[94]
CREB	Transfected mice with CREB300/310 dramatically enhances tumor growth, whereas CREB300/310/133 inhibits the growth of the implanted tumor cells	Abramovitch *et al*.[61]
E2F-1/RB1	Knockdown of E2F of mouse embryonic fibroblast leads to phosphorylation of RB1. The result is cell proliferation	Wu *et al*.[95]
ETS-1	Overexpression in a rat hind limb ischemia model led to angiogenesis by increasing HGF and VEGF	Hashiya *et al*.[96]
EWS/ETS	EWS/ETS fusion activated telomerase in Ewing's sarcoma cells and appears to activate the transcription of hTERT as a transactivator	Takahashi *et al*.[55]
FOX03a	*C. elegans* Daf16, an ortholog to FKHRL1, FKHR and AFX, is the major output of insulin signaling	Lee *et al*.[97]
HIF-1a/HIF-1b(ARNT)	Shown to enhance neovascularization in the rabbit ischemic hindlimb model	Vincent *et al*.[98]
Myc	Overexpression in mice resulted in downregulation of IL-6- and VEGF-induced rabbit corneal angiogenesis	Hatzi *et al*.[32]
NF-kB	Bortezomib has completed phase II trials where the NF-kB pathway was downregulated in refractory multiple myeloma and relapsed myeloma patients	Richardson *et al*.[99]
PEA3	Use of a dominant-negative PEA3 delayed onset, reduced number and size of mammary tumors in mice	Bieche *et al*.[75]
RARa	Downregulation of RARa resulted in lymphoma in 44% of homozygous transgenic mice	Manshouri *et al*.[100]
SP-1	Nude mice trial using celecoxib-affected Sp1-binding sites on VEGF gene expression and limited metastasis	Wei *et al*.[72]
STAT3	Nude mice xenografts provide a method of knocking down STAT3	Sun *et al*.[46]
STAT5	Downregulation of STAT5b mediates proliferation of SCCHN cancer cells	Leong *et al*.[101]
p53	Adenovirus-mediated wild-type *p53* gene transfer with chemotherapy and radiation therapy inhibits progression of lung cancer growth in animal models with minimal toxicity	Nishizaki *et al*.[102]

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