Chapter 21

Non-Androgen Regulated Transcription Factors as Novel Potential Targets for Prostate Cancer Therapy

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1. Introduction

1.1. Overview of prostate cancer and standard treatments

The estimated number of new prostate cancer cases for 2011 was 240,890. The majority of diagnosed prostate cancers (PCa) is found early due to the widespread use of the screening test for prostate specific antigen (PSA) and are considered low risk [1]. The prognosis for men diagnosed with low-risk prostate cancer is good and the NIH is recommending active surveillance [1]. Active surveillance has the benefit of reducing treatment side effects, including erectile dysfunction and incontinence, for men that are unlikely to die from their cancer [2]. Locally advanced prostate cancers are higher risk, and a substantial fraction of these patients will eventually die of the disease, though median survival may be as long as 5 years. If prostate cancer has spread to distant organs, current therapy will inevitably fail [3]. Because the androgen receptor (AR) is important for prostate cancer development and progression, androgen deprivation therapy (ADT), which either reduces the production of androgens by surgical or medical castration, or interferes with AR function via the use of antiandrogens, is increasingly becoming a central component in the management of metastatic prostate cancer [3]. ADT initially leads to improved clinical outcomes in about 90% of the cases. However, most tumors become androgen independent (AI) and no longer respond to standard hormonal therapies, chemotherapeutics or radiotherapy [3]. Thus, improved therapeutic strategies that target key pathways and molecules are essential to improve the outcome for patients with AI prostate cancer (AIPC). Interestingly, recent data shows that the AR pathway is often still engaged in AIPC, possibly due to receptor promiscuity or hypersensitivity. Therefore, some scientists believe that a strategy of targeting AR expression, ei-

ther directly or indirectly, may be helpful in these cases [4]. Indeed, elegant methods employing genome wide analysis are being used to identify small molecule antagonists of AR function [5]. Other ideas for targeted therapies include small molecule inhibition of metabolic enzymes such as fatty acid synthase (FASN) because cancer cells, unlike their normal counterparts, synthesize de novo large quantities of fatty acids and cholesterol [6] and inhibitors of vascular endothelial growth factor receptor (VEGFR) to suppress vascularization [7].

2. Non-androgen regulated transcription factors in prostate cancer; rationale for targeting

Most targeted small molecule therapies under development interfere with the function of receptors on the cell surface or kinases located in the cytoplasm. Transcription factors have been underutilized as targets of cancer therapeutics, the exceptions being the steroid hormone receptors, such as the AR, and nuclear factor kappa B (NF- ▪ B) [8, 9]. However, it is imperative to identify novel targets for the design of molecular treatments for cancers, including AIPC. Advances in drug delivery systems and a better understanding of how transcription factors act should overcome issues with targeting this important group of proteins. Thus, we believe that effective therapeutics for AIPC can be developed by identifying and targeting key transcriptional regulators, other than the AR, that are required for prostate cancer proliferation and survival. To identify potential targets that are master transcriptional regulators, one looks for DNA binding proteins whose activity is required for cell fate decisions, stem cell homeostasis, proliferation, and development. The regulatory roles played by Core Binding Factor (CBF) [10] and CBF1, Suppressor of Hairless, Lag-1 (CSL), the downstream effector of Notch receptors, place these transcription factors at the pinnacle of signaling cascades required for malignancy [11, 12]. Perhaps not surprisingly, these two pathways are genetically linked and exhibit cross talk. For example, enforced expression of RUNX1 rescues the Notch1-null phenotype in zebrafish [13] and in Notch1-null mice RUNX1 expression is greatly reduced [14]. Moreover, Notch and RUNX1 cooperate during T-cell specification in mammals and CBF is required for pre-thymic cells response to Notch signaling [15]. Thus, these two important transcriptional pathways are linked and, together, present a number of novel targets for the development of cancer therapies.

3. Core binding factor

More than twenty years ago, Nancy Speck and David Baltimore identified a DNA binding activity that bound to the core site (TGTGGTAA) in the enhancer of Moloney Murine Leukemia Virus that, when mutated, altered disease specificity to produce thymic leukemia instead of erythroleukemia [16, 17]. This DNA binding activity, which was named Core Binding Factor, was identified in a variety of cell lines [16]. Dr. Speck's laboratory purified several peptides that had core- binding activity from calf thymus nuclei [18]. The Speck laboratory then went on to sequence 5 peptides and used these sequences to isolate 3 cDNA

clones from a murine thymus library that encoded the three mammalian isoforms of CBFβ (CBFβ p22.0, CBFβ p21.5, and CBFβ p17.6). [19]. The Speck study demonstrated that CBFβ did not bind to DNA itself but, instead, partnered with a DNA binding protein, at that time termed acute myeloid leukemia-1 (AML-1), since one of their peptides appeared to be contained in the bovine homologue of the human AML-1. AML-1 had been identified by virtue of its involvement in the t(8;21) chromosomal translocation in 1991 [20]. A similar DNA binding activity was also isolated via interaction with the polyomavirus enhancer and was called polyomavirus enhancer binding protein 2 (PEBP2) [21]. CBF also binds to the Type B leukemogenic virus enhancer [22]. In 1993, Scott Hiebert's laboratory demonstrated that AML-1 selected a site related to the enhancer core motif (TGT/cGGT) and identified the DNA binding domain [23]. Later, Dr. Hiebert's group identified a larger isoform of AML-1 (termed AML-1B) produced from the *AML-1* gene using a homology screen of a human B-cell library [24]. Two other AML-1 family members expressed from independent genes were identified; AML-2 and AML-3 [25]. Following these studies, the AML-1 family of proteins underwent a revision in nomenclature with guidance from the Human Genome Organization [26]. AML-1 is now termed RUNX1, AML-2 is now termed RUNX3, and AML-3 is now termed RUNX2. The murine nomenclature is written in small case. This nomenclature will be used for the remainder of the chapter.

Mammalian CBF is a heterodimeric complex consisting of RUNX1, RUNX2, or RUNX3. As the Speck laboratory suggested, these three proteins bind to promoters and enhancers of target genes (or viral LTRs) as a heterodimer with CBFβ [10, 27]. DNA binding is achieved with a central domain (runt domain), consisting of an S-type immunoglobulin fold resembling the DNA binding domains of p53 and NF-kB [23, 28]. Although CBFβ does not contact DNA it regulates and enhances RUNX protein DNA binding via interactions with the Runt domain [28]. Complexity in CBF-regulated transcription comes about not only through co-expression in many tissues and a highly conserved DNA binding domain and recognition sequence, but also through the existence of multiple isoforms. For example, the *RUNX1* gene produces three main isoforms, all of which contain the DNA binding domain. These isoforms are thought to have both overlapping and unique functions. For example, RUNX1 isoforms are differentially expressed during hematopoietic differentiation of human embryonic stem cells (ESCs) and the RUNX1c isoform is expressed at the time of emergence of definitive HSCs [29]. Such complexity makes it difficult to assign function to each RUNX isoform and clearly, we are just at the beginning of understanding the distinct roles played by each protein. CBFβ is encoded on one gene in mammals but, as noted above, multiple isoforms are produced that may have distinct functions [19].

CBF is conserved in all multicellular organisms examined but is not present in yeast or any nonmetazoan studied to date. RUNX and CBFβ genes were identified in the nematode *C. elegans*, the fruit fly *Drosophila melanogaster*, which contains two CBFβ genes and four RUNX genes, the sea urchin (*Strongylocentrotus purpuratus*), sponges, puffer fish (*Takifugu rubripes)*, and the zebrafish (*Danio rerio*) [30-32]. In *Drosophila*, *RUNT*, the first RUNX gene identified in that organism, is required for segmentation [33]. *RUNT* gene mutations produce fly embryos with segmentation defects while *Lozenge*, a second RUNX gene in fruit flies, is required for eye development (Coffman 2009). In sea urchin, the *spRunt-1* gene is required

throughout development for cellular proliferation, cell survival, and tissue-specific gene expression [30]. Unlike mammals, two CBFβ homologs exist in *Drosophila. Big brother* and *Brother (Bgb* and *Bro)* display high homology to human CBFβ and are required for RUNX gene function in flies [34]. Studies in these model organisms have clearly demonstrated that CBF coordinates cellular proliferation, stem cell fate and terminal differentiation [30, 35].

Mouse genetics further demonstrate specific requirements for CBF in development and stem cell function. For example, RUNX1 is required for hematopoietic development and *Runx1* null animals die *in utero* by day E12.5 due to a complete absence of fetal-liver derived hematopoiesis [36]. Runx2 is critical for skeletal morphogenesis and *Runx2* null mice survive until birth but die shortly thereafter due to a complete lack of bone formation [37]. Interestingly, Runx1 and Runx3 are also expressed in bone cells and support skeletal development [27, 38]. *Runx3* null mice were reported to display gut hyperplasia due to an increase in cell proliferation and a reduced rate of apoptosis [39]. However, a second study showed that Runx3-deficient mice develop severe limb ataxia due to a defect in the dorsal root ganglion (DRG) proprioceptive neurons [40]. Runx3 is also important for hematopoiesis [27, 41]. Similar genetic studies demonstrated that CBFβ is required for RUNX protein function. For example, CBFβ knockdown mice recapitulate the *Runx1* null phenotype and hematopoietic-specific rescue of CBFβ null animals has demonstrated that CBFβ, like Runx2, is required for skeletal development [42, 43]. Thus, CBF functions as a master regulator of genes required for development, differentiation and stem cell maintenance [44, 45]. The requirement for CBFβ is likely due to it's ability to enhance RUNX DNA binding and, therefore, to augment the transcriptional strength of the RUNX factors [46].

4. Cancers associated with alterations to CBF

Alterations to CBF activity result in human disease. For example, human RUNX1 was first identified as the target of the t(8;21) chromosomal translocation associated with acute myelogenous leukemia (AML) [20, 47]. The t(8;21) is associated with approximately 12% of AML cases [48]. The t(8;21) results in the production of a chimeric transcription factor that retains the RUNX1 (chromosome 21) DNA binding domain but replaces the entire C-terminus with MTG8 (also called ETO), a transcriptional co-repressor [24, 49, 50]. RUNX1 is also the target of the rarer t(16;21) found in both de novo and therapy-related AML [51] and the t(12;21) identified in pre-B-cell acute lymphoblastic leukemia (ALL) [52]. These translocations fuse the RUNX1 DNA binding domain to an ETO-related protein termed MTG16 (CBFA2T3) and to an ETS-related transcription factor, respectively, to create chimeric gene regulatory factors [51, 52]. CBFβ is also targeted by genomic abnormalities that lead to AML. For example, the pericentric inversion of chromosome 16 produces a chimeric CBFβ/smooth muscle myosin heavy chain (SMMHC) protein termed CBFβ–SMMHC [53]. These chimeric transcription factors are thought to contribute to leukemogenesis by interfering with CBF-regulated transcription [54]. Moreover, these chromosomal abnormalities demonstrate that CBF alterations can result in both lymphoid and myeloid leukemias.

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CBF's role in blood development and in leukemia was brought into sharp focus by animal studies and by the identification of the molecular defects associated with AML. For many years, RUNX1 was considered blood specific, in part because of the strong phenotype obtained in *Runx1*-null mice. More recently, the expression, composition and function of CBF was studied in a wide variety of normal and cancerous cell lines and tissues. For example, RUNX protein expression was identified in the hair follicle stem cells (HFSCs) of the skin, and CBF is required to regulate HFSC proliferation [55]. Moreover, RUNX1 expression is activated in a chemical-induced model of rodent skin squamous cell carcinoma [55].

The expression of RUNX factors in prostate epithelial cell lines and normal prostate tissue was identified by real-time RT-PCR [56]. RUNX1, RUNX2, and RUNX3 were variously expressed in normal prostate tissue, an immortalized, non-transformed cell line, prostate cancer cell lines and primary prostate cancers [56]. To confirm that mRNA expression led to active DNA binding activity, CBF presence was confirmed using electrophoretic mobility shift assay (EMSA) [56]. While RUNX1 and RUNX2 were always expressed in prostate cancer cell lines, RUNX3 expression was not observed in most prostate cancer cell lines [56]. This correlates well with other studies that have identified RUNX2 expression in prostate cancer cell lines and showed that decreasing RUNX2 expression inhibits cell growth [57]. RUNX2 may play a role in tumor spread since RUNX2 triggers expression of bone-specific genes in prostate cancers, which may be involved in bone metastasis [58, 59]. Moreover, in a PTEN-deleted mouse model of prostate cancer, developing tumors increased Runx2 expression [60]. Thus, there is evidence that Runx2 expression is increased in malignant versus benign prostate tissue and is associated with tumor metastasis [61]. Interestingly, in a study of 314 patients with clinically localized prostate cancer that were treated with radical prostatectomy, the allelic variant RUNX1 rs2253319 was associated with metastasis to lymph nodes [62]. These data illustrate both the complexity of CBF expression in prostate and the involvement of CBF in cancer growth and metastasis. CBF is also highly expressed or altered in lung, endometrioid, and breast cancers [63-65].

CBF interacts with steroid hormones in various tissues. For example, the vitamin D receptor (VDR) associates with RUNX2 to regulate osteocalcin gene expression [66] and inappropriate expression of osteocalcin in prostate cancer cells depends upon RUNX2 [38]. CBF also interacts with the androgen receptor. RUNX1 and RUNX2 have both been shown to activate transcription from the prostate specific antigen (PSA) promoter and RUNX1 and RUNX2 physically associate with the AR [56] [57]. In prostate cancer cell lines, RUNX2 enhances TGF-β and androgen response [57]. Thus, the CBF and AR transcriptional pathways intersect in a way that enhances AR signaling. These data suggest that targeting CBF in prostate tumors should negatively impact AR signaling as well.

5. CBF inhibitors

Given that CBF and the AR pathways intersect and that CBF has been shown to regulate gene expression changes associated with tumorigenesis and metastasis in prostate cancer

cell lines, it seems reasonable to identify small molecules that can inhibit CBF function. Small molecules that interfere with the interaction between the RUNX proteins and CBFβ were recently described. In the first of these studies, the 3D structure of CBFβ was solved using NMR and the RUNX1 binding interface was determined [67]. This information was then used to perform a virtual chemical screen and using that information, allosteric inhibitors of CBFβ were identified. The most potent inhibitor, "17", inhibited proliferation of the ME-1 cell line, a line derived from a patient with acute myelomonocytic leukemia containing the inv(16), by about 40% and showed very little cytotoxicity [67]. Treatment of cells with 100 μm concentrations of Inhibitor 17 reduced RUNX1 DNA binding by about 30%. Thus, compound 17 binds to a site removed from the heterodimerization interface and produces moderate changes in CBF DNA binding and cellular proliferation. These data suggest that allosteric inhibitors of protein complex formation could be useful for probing CBF's role in cancer.

A recent approach to identify a role for CBF in prostate and ovarian cancer provides compelling evidence that CBF is a druggable target. Davis and co-workers showed that CBFβ - specific shRNAs inhibited the malignant phenotype of prostate and ovarian cancer cell lines [68]. Cell lines displaying 70% reduction in CBFβ were unable to grow in an anchorage independent manner and did not form xenograft tumors in mice. Gene array data (Agilent whole genome array) gathered during this study suggested that CBF-mediated gene expression was inhibited. Bioinformatic searches for RUNX DNA binding sites in the promoter regions of the differentially expressed genes revealed that of the 200 genes that exhibited altered expression, over 20% contained multiple putative RUNX binding sites (analyzed using the consensus TGT/CGGT) within their upstream regulatory regions [68]. EMSA was used to confirm a loss in CBF DNA binding activity [68]. These data clearly demonstrate that inhibition of CBFβ expression leads to a reduction in CBF activity and that CBF activity is required for the transformed phenotype.

The DNA binding activity of recombinant CBF is amenable to high throughput screening (HTS) assays and a recent screen of the NIH Clinical Collection Library has identified compounds that inhibit CBF (Davis and Meyers, unpublished data). The CBFβ siRNAs and compounds identified via HTS or virtual screens show promise as tools for discovery and as molecules that can be further developed into small molecule therapeutics in prostate cancer.

6. The Notch pathway

Notch gene mutations were first discovered in *Drosophila* via malformations of the wing [69]. This ligand-activated signaling pathway is a highly conserved mechanism for maintaining stem cell function and regulating apoptosis, proliferation and cell fate specification [69]. Mammals express four Notch receptor family members, termed Notch 1-4 and five ligands; two Jagged family ligands (jagged-1 and jagged-2) and three delta-like ligands (Dll1, Dll3 and Dll4) [69]. The Notch receptors are highly similar in structure and the extracellular domains contain epidermal growth factor-like repeats. The Notch li-

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gands are also transmembrane proteins. Thus, the Notch receptors regulate cell behavior via juxatacrine signaling that requires direct contact between the ligand-expressing cells and those cells expressing the receptor. Ligand binding activates two consecutive proteolytic cleavages to free the intracellular portion of the receptor, which is referred to as the Notch intracellular domain (NICD) [70]. The first cleavage is carried out by an A Disintegrin And Metalloprotease (ADAM)-family of transmembrane metalloproteases. The second cleavage is carried out by γ-secretase, an integral membrane enzyme complex, that is perhaps best known for its role in generating the amyloid-beta peptide found in brains of Alzheimer's disease patients [70, 71]. The NICD is a transcriptional co-activator. Once released, it travels to the nucleus via a nuclear transport signal where it binds to DNA-bound CSL. NICD binding to CSL displaces repressor complexes and recruits the mastermind family (MAML, mastermind like) of transcriptional coactivators, thereby activating the transcription of Notch-responsive genes [69]. In the absence of Notch receptor activation, CSL nucleates transcriptional repressive complexes via recruitment of histone deacetylase activities through interaction with SHARP (SMART and HDAC associated repressor) and corepressors like SMART/NcoR, CtIP/CtBP or ETO family members [72]. Interestingly, ETO (also called MTG8) is the target of the t(8;21) that produces a RUNX1/ETO fusion gene. Thus, the t(8;21) targets components of both the CBF and Notch pathways, highlighting yet another way in which these pathways intersect.

To date, a limited number of Notch-responsive genes have been identified. Some of the first gene targets identified include the transcription factors Hairy and enhancer of split-1 (Hes1) and Hairy and enhancer-of-split related with YRPW motif 1 (Hey1). Both Hes1 and Hey1 can be activated by a constitutively activated Notch1 receptor suggesting that these genes are bona fide targets [69]. Other CSL target genes are important mediators of signaling, including Akt and NF-κB, and important cell cycle regulators such as c-myc, D-type cyclins, $p21^{Waf1/Cip1}$ and p53 [69]. CSL is the only down-stream transcription factor directly responsive to Notch activation and, therefore, is crucial to Notch function.

The Notch pathway is deregulated in a variety of leukemias and solid cancers. For example, the mammalian orthologue of Notch was identified as TAN1 the target of the t(7;9)(q34;q34.4) in T-cell acute lymphocytic leukemia (T-ALL). While the t(7;9) is relatively rare (1% of all T-ALL) [73], the Notch1 receptor is constitutively activated by point mutations in the majority of T-ALL (almost 60%) [74]. Subsequent to the identification of Notch alterations in T-cell leukemia, the Notch pathway has been implicated in a variety of other human malignancies including cancers of the breast, ovarian, prostate, colorectal, and pancreas, as well as other leukemias [75-78]. In breast cancer, the Notch pathway components are commonly over-expressed and increased expression of Notch or Jag1 correlates with poor prognosis [76]. More recently, some studies suggest that breast cancer stem cell fate is regulated through the Notch pathway [79]. The Notch pathway is required for normal development of the murine prostate, and like breast cancers, prostate cancers also utilize the Notch pathway [80]. For example, Notch-1 and Jagged-1 expression constitute part of a gene expression signature for prostate cancer [81]. Other evidence indicating a role for Notch signaling in pros-

tate cancer includes studies showing that Jagged-1 expression correlates with prostate cancer recurrence and proliferation of prostate cancer cell lines [82, 83]. Moreover, down-regulation of both Notch-1 and Jagged-1 expression in the androgen insensitive prostate cancer cell line, PC3, was associated with a loss of malignancy and a reduction in Akt, mTOR and NF-κB activation [84].

As discussed above, the constitutive activation of Notch receptor signaling in diverse cancers is well documented, but the contribution of CSL to Notch-dependent oncogenesis has not been well studied. Our recent publication was the first to demonstrate that CSL was essential for the growth of prostate and breast cancer derived cell lines [85]. In these cancer cells, where Notch signaling is constitutive, CSL is required for growth *in vitro*. Thus, CSL is not only the focal point of Notch-dependent transcriptional control but appears to be central to the oncogenic Notch pathway as well [85].

In addition to the oncogenic functions associated with Notch signaling, the Notch pathway can also be tumor suppressive in cells or tissues where Notch predominately promotes differentiation [86]. Notch associated tumor suppressor activity is best illustrated in carcinoma of the skin, where keratinocyte specific inactivation of Notch1, Delta-like 1 (Dl1) or γ-secretase treatment accentuates tumor formation in chemical carcinogenesis models [87]. Increasingly, tumor suppressive activities of the Notch pathway are being reported, as interest in Notch signaling and the use of γ-secretase inhibitors to block Notch receptor activation has expanded. Inactivating mutations of Notch1 have been identified in head and neck squamous cell carcinoma [88] and haploinsufficiency of Notch1 or inhibition of Notch signaling with monoclonal antibodies to the Notch ligand Delta-like 4 induces vascular tumors in model systems [89]. As if to highlight the context dependent nature of Notch signaling, one report provided evidence that activated Notch1 alleles cooperated with oncogenic Ras to induce pancreatic cancer while a second report indicated that inactivation of Notch1 cooperated with Ras pathways in pancreatic cancers [90, 91]. This duality of function associated with Notch signaling has led to serious concerns regarding Notch receptor activation as a target of therapeutic intervention [86].

In prostate cancer, like in other cancers discussed above, Notch pathway signaling can be tumor suppressive. For example, NICD activity and Hes1 expression have been observed to be high in benign prostatic hyperplasia but low in prostate cancer indicating that Notch pathway activation can be lost during malignant transformation. Additionally, activation of the Notch pathway in the androgen independent prostate cancer cell line, DU145, inhibited cell growth and resulted in the activation of the PTEN tumor suppressor. Interestingly, knockdown of CSL in the DU145 cell line results in loss of cell growth (Yong and Davis, unpublished data). These data demonstrate that CSL (in a repressed complex) is required in cells where the Notch pathway can display tumor-suppressing activity. Clearly, the activity of the Notch pathway in prostate cancer is context dependent and complex.

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7. Notch pathway inhibitors

Regardless of the data implicating the Notch pathway in tumor suppression as well as oncogenesis, chemotherapeutic targeting of the Notch pathway employing γ-secretase inhibitors (GSI) to block release of the NICD has generated much interest [92]. GSIs, which were designed primarily for Alzheimer's disease, developed by Merck, Novartis, Pfizer and Roche are currently in clinical trials for a number of malignancies including T-ALL, lymphoma, breast, colorectal, brain, pancreatic, and non-small cell lung carcinoma. However, targeting the Notch pathway through the use of GSIs is problematic. Preclinical studies examining GSI function *in vitro* are difficult because, with the notable exception of GSI-1, these drugs do not display strong inhibitory effects on cell growth or survival *in vitro*. Also, while these drugs do inhibit Notch signaling, they display poor specificity. As an example, the inhibition of survival of breast carcinoma cell lines by GSI-1 was associated with inhibition of the proteosome and not effects on Notch signaling [93]. In addition to off-target effects, Notch inhibition by GSI has adverse effects on the intestinal system and immune function [94]. Lastly, as discussed above, the cell context determines whether the Notch pathway is oncogenic or tumor suppressive even within cancers of the same organ [86]. Thus, the consequence of inhibition of Notch receptor activation by GSI or inhibitory antibodies to Notch receptors/ligands is difficult to predict.

Inhibition of Notch activation by GSIs, inhibitory antibodies that bind DSL ligands, or other inhibitors of receptor activation target only the Notch activated state and they are less than ideal. However, the Notch pathway is central to oncogenesis, and this idea fuels the search for novel ways to inhibit the Notch signaling pathway [11]. Recently, the Bradner laboratory developed a stabilized peptide that mimics MAML and binds to the NICD-CSL complex to block interaction with endogenous MAML [95]. SAHM1, a 16 amino acid peptide which blocks MAML binding to the NICD-CSL complex is cell-permeable and lowers NOTCH-target gene expression when added to cells in culture [95]. SAHM1 lowers proliferation of T-ALL cell lines suggesting that these small molecules will be useful as probes to dissect the requirement for MAML in Notch signaling and as building blocks for a new generation of Notch inhibitors.

Davis and co-workers tested the idea that direct inhibition of CSL would not only abrogate Notch pathways in the activated oncogenic state, but also disrupt the transcriptional regulation of Notch pathway genes that are repressed in the Notch quiescent state [85]. According to this argument, in cells or tissues where Notch activation is tumor suppressive, inhibition of CSL would release the strong transcriptional repressive complexes positioned on Notch targets. Removal of CSL-dependent repressive complexes could mimic the tumor suppressive activity of the Notch pathway. Indeed, Davis and co-workers addressed the role of CSL in Notch-dependent signaling in prostate cancer cell lines, using lentiviral mediated transfer of shRNA specific for CSL to knockdown expression of CSL. CSL knockdown was tracked by EMSA and expression of the Notch pathway genes was documented using RT-PCR array profiling. Knockdown of CSL expression produced gene expression changes distinct from those induced by GSI inhibition of Notch signaling [85]. For example, inhibition of Notch

receptor activation by DAPT resulted in repression of Hes1, a well-characterized CSL target in prostate and breast cancer cell lines. In contrast, Hes1 mRNA levels were unaffected by CSL ablation in prostate cancer cell lines, indicating that Hes1 expression does not require the activating function of CSL [85]. Thus, Notch pathway-dependent transcriptional regulation of Hes1 is primarily through repression and ablation of CSL partially mimics Notch receptor activation. While HES1 expression was not significantly altered by CSL knockdown, the expression of other Notch pathway genes did change. One such gene, DTX1 is thought to regulate Notch signaling either by targeting the NICD for ubiquitination and degradation or by altering NICD transcriptional functions, possibly by competing for co-activators [96]. Davis and coworkers failed to generate stable cell lines after infection with the CSL-specific shRNA but not with the control non-target (NT) shRNA. CSL knockdown cells were poorly attached and growth inhibited as compared to the NT infected cells [96]. These data provide strong evidence that CSL, the major Notch pathway effector, is required for cell growth in prostate cancer cells lines, and suggest that CSL is an important candidate for small molecule therapies in AIPC.

8. Summary and future directions

Although the AR is an important target of therapeutics in the struggle against prostate cancer, it remains imperative to develop effective strategies to target other important transcirption pathways, especially in AIPC. To alter gene transcription, some scientists, for example, are developing histone acetyl transferase inhibitors [97]. However, any such therapeutic would be expected to lack specificity for particular oncogenic pathways. DNA binding transcription factors represent druggable targets that should produce a more specific outcome, and are under appreciated as targets of small molecule inhibitors. Master transcriptional regulatory factors such as CBF and CSL clearly play important roles in cancer cell biology. Numerous studies show that inhibiting their function results in cancer cell death or loss of malignancy. These may be particularly useful targets in prostate cancers as the pathways intersect and CBF enhances AR function. In the case of CBF, it may make sense to target CBFβ to inhibit CBF activity in cancers since the activity of CBF is clearly oncogenic, while individual RUNX proteins can act either as oncogenes or tumor suppressors [10]. Developing inhibitors against these key transcriptional regulators will allow their use not only for therapy but also as probes to understand specific transcriptional pathways that support cancer growth, proliferation and metastasis.

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Abbreviations

ADT: androgen deprivation therapy

AI: androgen independent

AIPC: androgen independent prostate cancer

ALL: acute lymphoblastic leukemia

AR: androgen receptor

CBF: Core Binding Factor

DRG: dorsal root ganglion

ECS: embryonic stem cells

GSI: γ-secretase inhibitors

NT: non-target

PCa: prostate cancer

PSA: prostate specific antigen

VEGFR: vascular endothelial growth factor receptor

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References

[1] Brawley, O.W., Prostate cancer epidemiology in the United States. World J Urol, 2012. 30(2): p. 195-200.

[2] Singer, E.A., et al., Active surveillance for prostate cancer: past, present and future. Curr Opin Oncol, 2012. 24(3): p. 243-50.

[3] Bluemn, E.G. and P.S. Nelson, The androgen/androgen receptor axis in prostate cancer. Curr Opin Oncol, 2012. 24(3): p. 251-7.

[4] Shiota, M., A. Yokomizo, and S. Naito, Increased androgen receptor transcription: a cause of castration-resistant prostate cancer and a possible therapeutic target. J Mol Endocrinol, 2011. 47(1): p. R25-41.

[5] Zhu, Z., et al., Dose-dependent effects of small-molecule antagonists on the genomic landscape of androgen receptor binding. BMC Genomics, 2012. 13(1): p. 355.

[6] Flavin, R., G. Zadra, and M. Loda, Metabolic alterations and targeted therapies in prostate cancer. J Pathol, 2011. 223(2): p. 283-94.

[7] Kluetz, P.G., W.D. Figg, and W.L. Dahut, Angiogenesis inhibitors in the treatment of prostate cancer. Expert Opin Pharmacother, 2010. 11(2): p. 233-47.

[8] Moretti, M., et al., Cancer: NF-kappaB regulates energy metabolism. Int J Biochem Cell Biol, 2012.

[9] Haddad, H. and J.A. Garcia, Novel agents for the management of castration-resistant prostate cancer. Curr Opin Urol, 2012. 22(3): p. 175-82.

[10] Cameron, E.R. and J.C. Neil, The Runx genes: lineage-specific oncogenes and tumor suppressors. Oncogene, 2004. 23(24): p. 4308-14.

[11] Groth, C. and M.E. Fortini, Therapeutic approaches to modulating Notch signaling: current challenges and future prospects. Semin Cell Dev Biol, 2012. 23(4): p. 465-72.

[12] Martinez Arias, A., V. Zecchini, and K. Brennan, CSL-independent Notch signalling: a checkpoint in cell fate decisions during development? Curr Opin Genet Dev, 2002. 12(5): p. 524-33.

[13] Nakagawa, M., et al., AML1/Runx1 rescues Notch1-null mutation-induced deficiency of para-aortic splanchnopleural hematopoiesis. Blood, 2006. 108(10): p. 3329-34.

[14] Kumano, K., et al., Notch1 but not Notch2 is essential for generating hematopoietic stem cells from endothelial cells. Immunity, 2003. 18(5): p. 699-711.

[15] Guo, Y., et al., Core binding factors are necessary for natural killer cell development and cooperate with Notch signaling during T-cell specification. Blood, 2008. 112(3): p. 480-92.

[16] Speck, N.A. and D. Baltimore, Six distinct nuclear factors interact with the 75-base-pair repeat of the Moloney murine leukemia virus enhancer. Mol Cell Biol, 1987. 7(3): p. 1101-10.

[17] Speck, N.A., B. Renjifo, and N. Hopkins, Point mutations in the Moloney murine leukemia virus enhancer identify a lymphoid-specific viral core motif and 1,3-phorbol myristate acetate-inducible element. J Virol, 1990. 64(2): p. 543-50.

[18] Wang, S.W. and N.A. Speck, Purification of core-binding factor, a protein that binds the conserved core site in murine leukemia virus enhancers. Mol Cell Biol, 1992. 12(1): p. 89-102.

[19] Wang, S., et al., Cloning and characterization of subunits of the T-cell receptor and murine leukemia virus enhancer core-binding factor. Mol Cell Biol, 1993. 13(6): p. 3324-39.

[20] Miyoshi, H., et al., t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. Proc Natl Acad Sci U S A, 1991. 88(23): p. 10431-4.

[21] Bae, S.C., et al., Isolation of PEBP2 alpha B cDNA representing the mouse homolog of human acute myeloid leukemia gene, AML1. Oncogene, 1993. 8(3): p. 809-14.

[22] Mertz, J.A., et al., Type B leukemogenic virus has a T-cell-specific enhancer that binds AML-1. J Virol, 2001. 75(5): p. 2174-84.

[23] Meyers, S., J.R. Downing, and S.W. Hiebert, Identification of AML-1 and the (8;21) translocation protein (AML-1/ETO) as sequence-specific DNA-binding proteins: the runt homology domain is required for DNA binding and protein-protein interactions. Mol Cell Biol, 1993. 13(10): p. 6336-45.

[24] Meyers, S., N. Lenny, and S.W. Hiebert, The t(8;21) fusion protein interferes with AML-1B-dependent transcriptional activation. Mol Cell Biol, 1995. 15(4): p. 1974-82.

[25] Levanon, D., et al., AML1, AML2, and AML3, the human members of the runt domain gene-family: cDNA structure, expression, and chromosomal localization. Genomics, 1994. 23(2): p. 425-32.

[26] van Wijnen, A.J., et al., Nomenclature for Runt-related (RUNX) proteins. Oncogene, 2004. 23(24): p. 4209-10.

[27] Cohen, M.M., Jr., Perspectives on RUNX genes: an update. Am J Med Genet A, 2009. 149A(12): p. 2629-46.

[28] Zhang, L., et al., Structural and functional characterization of Runx1, CBF beta, and CBF beta-SMMHC. Blood Cells Mol Dis, 2003. 30(2): p. 147-56.

[29] Challen, G.A. and M.A. Goodell, Runx1 isoforms show differential expression patterns during hematopoietic development but have similar functional effects in adult hematopoietic stem cells. Exp Hematol, 2010. 38(5): p. 403-16.

[30] Coffman, J.A., Is Runx a linchpin for developmental signaling in metazoans? J Cell Biochem, 2009. 107(2): p. 194-202.

[31] Rennert, J., et al., The evolution of Runx genes I. A comparative study of sequences from phylogenetically diverse model organisms. BMC Evol Biol, 2003. 3: p. 4.

[32] Robertson, A.J., et al., The evolution of Runx genes II. The C-terminal Groucho recruitment motif is present in both eumetazoans and homoscleromorphs but absent in a haplosclerid demosponge. BMC Res Notes, 2009. 2: p. 59.

[33] Kania, M.A., et al., The Drosophila segmentation gene runt encodes a novel nuclear regulatory protein that is also expressed in the developing nervous system. Genes Dev, 1990. 4(10): p. 1701-13.

[34] Golling, G., et al., Drosophila homologs of the proto-oncogene product PEBP2/CBF beta regulate the DNA-binding properties of Runt. Mol Cell Biol, 1996. 16(3): p. 932-42.

[35] Wang, C.Q., et al., Runx family genes, niche, and stem cell quiescence. Blood Cells Mol Dis, 2010. 44(4): p. 275-86.

[36] Okuda, T., et al., AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell, 1996. 84(2): p. 321-30.

[37] Komori, T., et al., Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell, 1997. 89(5): p. 755-64.

[38] Lian, J.B., et al., Regulatory controls for osteoblast growth and differentiation: role of Runx/Cbfa/AML factors. Crit Rev Eukaryot Gene Expr, 2004. 14(1-2): p. 1-41.

[39] Li, Q.L., et al., Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell, 2002. 109(1): p. 113-24.

[40] Levanon, D., et al., The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. EMBO J, 2002. 21(13): p. 3454-63.

[41] Inoue, K., T. Shiga, and Y. Ito, Runx transcription factors in neuronal development. Neural Dev, 2008. 3: p. 20.

[42] Wang, Q., et al., The CBFbeta subunit is essential for CBFalpha2 (AML1) function in vivo. Cell, 1996. 87(4): p. 697-708.

[43] Kundu, M., et al., Cbfbeta interacts with Runx2 and has a critical role in bone development. Nat Genet, 2002. 32(4): p. 639-44.

[44] Coffman, J.A., Runx transcription factors and the developmental balance between cell proliferation and differentiation. Cell Biol Int, 2003. 27(4): p. 315-24.

[45] Kurosaka, H., et al., Core binding factor beta functions in the maintenance of stem cells and orchestrates continuous proliferation and differentiation in mouse incisors. Stem Cells, 2011. 29(11): p. 1792-803.

[46] Tang, Y.Y., et al., Energetic and functional contribution of residues in the core binding factor beta (CBFbeta) subunit to heterodimerization with CBFalpha. J Biol Chem, 2000. 275(50): p. 39579-88.

[47] Erickson, P., et al., Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to Drosophila segmentation gene, runt. Blood, 1992. 80(7): p. 1825-31.

[48] Lam, K. and D.E. Zhang, RUNX1 and RUNX1-ETO: roles in hematopoiesis and leukemogenesis. Front Biosci, 2012. 17: p. 1120-39.

[49] Davis, J.N., L. McGhee, and S. Meyers, The ETO (MTG8) gene family. Gene, 2003. 303: p. 1-10.

[50] Miyoshi, H., et al., The t(8;21) translocation in acute myeloid leukemia results in production of an AML1-MTG8 fusion transcript. EMBO J, 1993. 12(7): p. 2715-21.

[51] Gamou, T., et al., The partner gene of AML1 in t(16;21) myeloid malignancies is a novel member of the MTG8(ETO) family. Blood, 1998. 91(11): p. 4028-37.

[52] Golub, T.R., et al., Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. Proc Natl Acad Sci U S A, 1995. 92(11): p. 4917-21.

[53] Liu, P., et al., Fusion between transcription factor CBF beta/PEBP2 beta and a myosin heavy chain in acute myeloid leukemia. Science, 1993. 261(5124): p. 1041-4.

[54] Hart, S.M. and L. Foroni, Core binding factor genes and human leukemia. Haematologica, 2002. 87(12): p. 1307-23.

[55] Hoi, C.S., et al., Runx1 directly promotes proliferation of hair follicle stem cells and epithelial tumor formation in mouse skin. Mol Cell Biol, 2010. 30(10): p. 2518-36.

[56] Fowler, M., et al., RUNX1 (AML-1) and RUNX2 (AML-3) cooperate with prostate-derived Ets factor to activate transcription from the PSA upstream regulatory region. J Cell Biochem, 2006. 97(1): p. 1-17.

[57] van der Deen, M., et al., The cancer-related Runx2 protein enhances cell growth and responses to androgen and TGFbeta in prostate cancer cells. J Cell Biochem, 2010. 109(4): p. 828-37.

[58] Stein, G.S., et al., Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. Oncogene, 2004. 23(24): p. 4315-29.

[59] Pratap, J., J.B. Lian, and G.S. Stein, Metastatic bone disease: role of transcription factors and future targets. Bone, 2011. 48(1): p. 30-6.

[60] Lim, M., et al., Runx2 regulates survivin expression in prostate cancer cells. Lab Invest, 2010. 90(2): p. 222-33.

[61] Akech, J., et al., Runx2 association with progression of prostate cancer in patients: mechanisms mediating bone osteolysis and osteoblastic metastatic lesions. Oncogene, 2010. 29(6): p. 811-21.

[62] Huang, S.P., et al., Clinical significance of runt-related transcription factor 1 polymorphism in prostate cancer. BJU Int, 2011. 107(3): p. 486-92.

[63] Tandon, M., et al., Runx2 mediates epigenetic silencing of the bone morphogenetic protein-3B (BMP-3B/GDF10) in lung cancer cells. Mol Cancer, 2012. 11: p. 27.

[64] CBFB Mutations and MAGI3-AKT3 Fusions Recur In Breast Cancer. Cancer Discov, 2012. 2(8): p. OF10.

[65] Planaguma, J., et al., A differential gene expression profile reveals overexpression of RUNX1/AML1 in invasive endometrioid carcinoma. Cancer Res, 2004. 64(24): p. 8846-53.

[66] Paredes, R., et al., Bone-specific transcription factor Runx2 interacts with the 1alpha, 25-dihydroxyvitamin D3 receptor to up-regulate rat osteocalcin gene expression in osteoblastic cells. Mol Cell Biol, 2004. 24(20): p. 8847-61.

[67] Gorczynski, M.J., et al., Allosteric inhibition of the protein-protein interaction between the leukemia-associated proteins Runx1 and CBFbeta. Chem Biol, 2007. 14(10): p. 1186-97.

[68] Davis, J.N., et al., Association of core-binding factor beta with the malignant phenotype of prostate and ovarian cancer cells. J Cell Physiol, 2010. 225(3): p. 875-87.

[69] Borggrefe, T. and F. Oswald, The Notch signaling pathway: transcriptional regulation at Notch target genes. Cell Mol Life Sci, 2009. 66(10): p. 1631-46.

[70] Kaether, C., C. Haass, and H. Steiner, Assembly, trafficking and function of gamma-secretase. Neurodegener Dis, 2006. 3(4-5): p. 275-83.

[71] van Tetering, G. and M. Vooijs, Proteolytic cleavage of Notch: "HIT and RUN". Curr Mol Med, 2011. 11(4): p. 255-69.

[72] Oswald, F., et al., SHARP is a novel component of the Notch/RBP-Jkappa signalling pathway. EMBO J, 2002. 21(20): p. 5417-26.

[73] Ellisen, L.W., et al., TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. Cell, 1991. 66(4): p. 649-61.

[74] Weng, A.P., et al., Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science, 2004. 306(5694): p. 269-71.

[75] Pece, S., et al., Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. J Cell Biol, 2004. 167(2): p. 215-21.

[76] Reedijk, M., et al., High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. Cancer Res, 2005. 65(18): p. 8530-7.

[77] Park, J.T., et al., Notch3 gene amplification in ovarian cancer. Cancer Res, 2006. 66(12): p. 6312-8.

[78] Reedijk, M., et al., Activation of Notch signaling in human colon adenocarcinoma. Int J Oncol, 2008. 33(6): p. 1223-9.

[79] Sharma, A., et al., A monoclonal antibody against human Notch1 ligand-binding domain depletes subpopulation of putative breast cancer stem-like cells. Mol Cancer Ther, 2012. 11(1): p. 77-86.

[80] Whelan, J.T., et al., Notch-1 signaling is lost in prostate adenocarcinoma and promotes PTEN gene expression. J Cell Biochem, 2009. 107(5): p. 992-1001.

[81] Kong, D., et al., Cancer Stem Cells and Epithelial-to-Mesenchymal Transition (EMT)-Phenotypic Cells: Are They Cousins or Twins? Cancers (Basel), 2011. 3(1): p. 716-729.

[82] Zhang, Y., et al., Down-regulation of Jagged-1 induces cell growth inhibition and S phase arrest in prostate cancer cells. Int J Cancer, 2006. 119(9): p. 2071-7.

[83] Santagata, S., et al., JAGGED1 expression is associated with prostate cancer metastasis and recurrence. Cancer Res, 2004. 64(19): p. 6854-7.

[84] Wang, Z., et al., Down-regulation of Notch-1 and Jagged-1 inhibits prostate cancer cell growth, migration and invasion, and induces apoptosis via inactivation of Akt, mTOR, and NF-kappaB signaling pathways. J Cell Biochem, 2010. 109(4): p. 726-36.

[85] Yong, T., et al., Down regulation of CSL activity inhibits cell proliferation in prostate and breast cancer cells. J Cell Biochem, 2011. 112(9): p. 2340-51.

[86] Lobry, C., P. Oh, and I. Aifantis, Oncogenic and tumor suppressor functions of Notch in cancer: it's NOTCH what you think. J Exp Med, 2011. 208(10): p. 1931-5.

[87] Nicolas, M., et al., Notch1 functions as a tumor suppressor in mouse skin. Nat Genet, 2003. 33(3): p. 416-21.

[88] Stransky, N., et al., The mutational landscape of head and neck squamous cell carcinoma. Science, 2011. 333(6046): p. 1157-60.

[89] Ryeom, S.W., The cautionary tale of side effects of chronic Notch1 inhibition. J Clin Invest, 2011. 121(2): p. 508-9.

[90] Hanlon, L., et al., Notch1 functions as a tumor suppressor in a model of K-ras-induced pancreatic ductal adenocarcinoma. Cancer Res, 2010. 70(11): p. 4280-6.

[91] De La, O.J., et al., Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. Proc Natl Acad Sci U S A, 2008. 105(48): p. 18907-12.

[92] Takebe, N., et al., Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. Nat Rev Clin Oncol, 2011. 8(2): p. 97-106.

[93] Han, J., et al., The cytotoxicity of gamma-secretase inhibitor I to breast cancer cells is mediated by proteasome inhibition, not by gamma-secretase inhibition. Breast Cancer Res, 2009. 11(4): p. R57.

[94] Imbimbo, B.P., et al., Therapeutic intervention for Alzheimer's disease with gamma-secretase inhibitors: still a viable option? Expert Opin Investig Drugs, 2011. 20(3): p. 325-41.

[95] Moellering, R.E., et al., Direct inhibition of the NOTCH transcription factor complex. Nature, 2009. 462(7270): p. 182-8.

[96] Yamamoto, N., et al., Role of Deltex-1 as a transcriptional regulator downstream of the Notch receptor. J Biol Chem, 2001. 276(48): p. 45031-40.

[97] Perry, A.S., et al., The epigenome as a therapeutic target in prostate cancer. Nat Rev Urol, 2010. 7(12): p. 668-80.

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