Cell Cycle 9:15, 2986-2995; August 1, 2010; © 2010 Landes Bioscience

New tricks from an old oncogene

Gene fusion and copy number alterations of *MYB* in human cancer

Göran Stenman,* Mattias K. Andersson and Ywonne Andrén

Lundberg Laboratory for Cancer Research; Department of Pathology; The Sahlgrenska Academy at University of Gothenburg; Gothenburg, Sweden

Key words: MYB, NFIB, transcription factor, gene fusion, microRNA, gene duplication, adenoid cystic carcinoma, T-cell acute lymphocytic leukemia, breast cancer

Abbreviations: ETS, E26 transformation specific; HGPIN, high-grade prostatic intraepithelial neoplasia; CDKI, cyclin dependent kinase inhibitor; ACC, adenoid cystic carcinoma; UTR, untranslated region; miRNA, microRNA; CREB, cAMP response element-binding protein; AMV, avian myeloblastosis virus; NRD, negative regulatory domain; DBD, DNA binding domain; TAD, transactivation domain; ERα, estrogen receptor-α; T-ALL, T-cell acute lymphoblastic leukemia; TCRB, T-cell receptor beta; ODN, oligodeoxinucleotides; TKI, tyrosine kinase inhibitor; HDAC, histone deacetylase; DAPI, 4',6'-diamidino-2'-phenylindole dihydrochloride; cen, centromere; tel, telomere

Submitted: 05/20/10

Accepted: 05/28/10

Previously published online: www.landesbioscience.com/journals/cc/article/12515

DOI: 10.4161/cc.9.15.12515

*Correspondence to: Göran Stenman; Email: goran.stenman@llcr.med.gu.se

MYB is a leucine zipper transcription factor that is essential for hematopoesis and for renewal of colonic crypts. There is also ample evidence showing that MYB is leukemogenic in several animal species. However, it was not until recently that clear evidence was presented showing that *MYB* actually is an oncogene rearranged in human cancer. In a recent study, a novel mechanism of activation of *MYB* involving gene fusion was identified in carcinomas of the breast and head and neck. A t(6;9) translocation was shown to generate fusions between *MYB* and the transcription factor gene *NFIB*. The fusions consistently result in loss of the 3'-end of *MYB*, including several highly conserved target sites for microRNAs that negatively regulate *MYB* expression. Deletion of these target sites may disrupt the repression of *MYB*, leading to overexpression of MYB-NFIB transcripts and protein and to transcriptional activation of critical *MYB* target genes associated with apoptosis, cell cycle control, cell growth/angiogenesis and cell adhesion. This study, together with previous and recent data showing rearrangements and copy number alterations of the *MYB* locus in T-cell leukemia and certain solid tumors, will be the main focus of this review.

©2010 Landes Bioscience. Do not distribute

Introduction: Fusion Oncogenes in Solid Tumors

Tumor-type specific chromosome rearrangements often result in gene fusions encoding potent oncoproteins. To date, close to 400 gene fusions have been identified in human cancer and an increasing number of these fusions are being recognized as important diagnostic and prognostic markers and as targets for new cancer therapies.[1,2] Recent estimates have indicated that gene fusions account for 20% of human cancer morbidity. Most gene fusions have thus far been identified in leukemias and sarcomas and only a few in carcinomas.[1] This paucity is likely due to an inability to discover these rearrangements rather than a true lack of gene fusions in carcinomas. Recent data suggest that the mechanisms by which they are generated may be partly different from that in leukemias and sarcomas. Thus, previous cytogenetic studies have clearly demonstrated that recurrent balanced chromosome translocations are rare in carcinomas whereas they are common in leukemias and sarcomas.[2] Therefore, it is not surprising that several new gene fusions in carcinomas have been shown to result from intrachromosomal rearrangements or cryptic translocations/insertions that are not visible at the cytogenetic level.[3-7] In line with this reasoning, it was recently shown that more than 50% of prostate cancers harbor androgen regulated gene fusions in which an *ETS* (E26 transformation specific) transcription factor gene (*ERG* or *ETV1*) is fused to the *TMPRSS2* promoter region,[4] and subgroups of non-small-cell lung cancers have *EML4-ALK*, *SLC34A2-ROS* or *CD74-ROS* gene fusions.[5,8] In addition, fusion oncogenes have been found in subsets of thyroid carcinomas, renal cell carcinomas, breast cancer and mucoepidermoid carcinomas of the salivary and bronchial glands.[1,9,10] Continued studies of cancer genomes using massively parallel

sequencing and paired-end sequencing strategies will most likely lead to the identification of new gene fusions also in other common epithelial cancers.

Fusion genes are potent oncogenes as demonstrated by their ability to induce tumors in various transgenic mouse models. For example, the *RET-PTC1* fusion induces tumors that are morphologically similar to human papillary thyroid carcinomas when specifically overexpressed in the thyroid.[11,12] Similarly, the *FUS-DDIT3* fusion causes myxoid liposarcomas when expressed under control of a ubiquitously expressed promoter,[13] the *SYT-SSX2* fusion induces synovial sarcoma-like tumors when expressed in immature myoblasts,[14] whereas the *PAX3-FKHR* fusion induces alveolar rhabdomyosarcomas when targeted to *Myf6*-expressing skeletal muscle cells.[15] In the latter mice, the incidence of tumors is significantly increased by concurrent loss of *p53* or *Ink4a/Arf* function.

Recent studies have also shown that the *ETV6-NTRK3* fusion, typical of human secretory breast carcinoma, can induce breast tumors in mice through transformation of committed alveolar bipotent or CD61(+) luminal progenitor cells,[16] and that the *EML4-ALK* fusion induces lung adenocarcinomas in mice.[17] Similarly, studies of the oncogenic role of the *TMPRSS2-ERG* fusion have shown that ERG has an important role in mouse prostate cancer progression and cooperates with *Pten* haploinsufficiency to promote progression of high-grade prostatic intraepithelial neoplasia (HGPIN) to invasive prostatic adenocarcinoma and that transgenic *TMPRSS2-ERG* mice develop PIN, but only in the context of PI3-kinase pathway activation.[18,19] Taken together, these studies clearly demonstrate that fusion oncogenes are pathogenetically critical genes in solid tumors and as such also key targets for therapy.

The majority of fusion oncogenes in solid tumors encode aberrant transcription factors while a minority express chimeric proteins that deregulate growth factor signaling.[20] Many fusion oncoproteins exert their effects through deregulation of genes encoding cell cycle proteins or by affecting the stability of these proteins. For example, the Ewing sarcoma associated EWSR1-FLI1 fusion protein has been shown to induce expression of *MYC*, *CCND1* and *CCNE1* both at the mRNA and protein levels[21-23] and also to downregulate *CDKN1A* (p21), *CDKN1B* (p27) and *CDKN1C* (p57), leading to a reduction of the corresponding CDKI (cyclin dependent kinase inhibitor) proteins.[21,22,24] Recently, Sanchez et al. also demonstrated that EWS-FLI1 upregulates a more oncogenic isoform of cyclin D1, termed D1b, by promoting the expression of an alternative splice form of the *CCND1* transcript.[25] Cyclin D1 is also induced by several other fusion oncoproteins such as ETV6-NTRK3, SYT-SSX2 and RET-PTC1.[26-28] The PAX3-FKHR fusion protein has been shown to increase proteasome-dependent degradation of p27,[29] and the FUS-DDIT3 fusion protein was recently shown to directly bind CDK2 through its DDIT3 part.[30] However, the biological consequence of this interaction is not yet fully understood.

Gene Fusions Involving Transcription Factors and Transcriptional Coactivators in Salivary Gland Tumors

©2010 Landes Bioscience. Do not distribute.

Tumors of the salivary glands constitute up to 10% of the tumors of the head and neck region.[31] There are more than 30 histopathological subtypes, of which pleomorphic adenomas and Warthin tumors are the most common benign tumors and mucoepidermoid carcinomas and adenoid cystic carcinomas (ACC) are the most common malignant tumors.[32] Recent studies have shown that these tumor types are characterized by a highly specific pattern of chromosome translocations resulting in fusion oncogenes.[3,6,7,9,33,34] The major targets of the translocations are transcription factors involved in growth factor signaling and cell cycle regulation, transcriptional coactivators and tyrosine kinase receptors.

Pleomorphic adenomas are characterized by translocations resulting in gene fusions involving the transcription factor genes *PLAG1* and *HMGA2*. PLAG1 is a developmentally regulated DNA-binding zinc finger protein that belongs to a family of cell cycle progression-related proteins (reviewed in ref. 9 and 35). Ectopic overexpression of *PLAG1* due to promoter swapping with at least five other genes (**Fig. 1A**), cause deregulation of PLAG1 target genes and activation of the IGF-II mitogenic signaling pathway in salivary gland pleomorphic adenomas.[36] *PLAG1* is also involved in gene fusions with two other genes (*HAS2* and *COL1A2*) in lipoblastomas (reviewed in ref. 9) and has been shown to induce acute myeloid leukemia in cooperation with the CBFB-MYH11 fusion protein in mice.[37]

HMGA2 belongs to a family of non-histone nuclear proteins that orchestrate the assembly of nucleoprotein complexes (reviewed in ref. 38). They play important roles in gene transcription, recombination and chromatin structure. Genomic rearrangements of the 3'-part of *HMGA2* due to gene fusions (**Fig. 1A**) or truncations in pleomorphic adenomas and benign mesenchymal tumors result in activation of the expression of *HMGA2* and its cellular targets, including the cell cycle regulators *CCNA1* and *CCNB2*.[39,40] In a subset of tumors with *HMGA2* fusions the gene fusion is amplified together with other closely linked genes such as *MDM2*.[7] The molecular mechanism by which *HMGA2* is activated by chromosomal rearrangements is still unclear. Previous studies have suggested that separation of the DNA-binding domains from the acidic domain and the mRNA-destabilizing AUUUA motifs in the 3'-UTR (untranslated region) is the critical event leading to stabilization and overexpression of *HMGA2* transcripts (reviewed in ref. 9). However, recent studies have indicated that HMGA2 may also be deregulated by loss of negatively regulating Let-7 microRNA (miRNA) target-sites in the 3'-UTR as a consequence of gene fusion/truncation.[41-43]

Fusion of the transcriptional coactivators MAML2 and CRTC1 (a.k.a. MECT1, TORC1 or WAMTP1) is a characteristic feature of low-grade mucoepidermoid carcinomas of the salivary, bronchial and thyroid glands (**Fig. 1B**).[44-46] MAML2 belongs to a family of Mastermind-like, nuclear proteins that functions as coactivators for Notch receptors[47,48] whereas CRTC1 belongs to a family of highly conserved CREB (cAMP response element-binding protein) coactivators.[49,50] The *CRTC1-MAML2* fusion encodes a chimeric protein consisting of the CREB-binding

domain of CRTC1 fused to the transactivation domain of MAML2.[44,45] Functional studies have shown that the N-terminal CREB-binding domain of the fusion is crucial for its transforming activity,[51,52] and that CRTC1-MAML2 can activate transcription of cAMP/CREB target genes (Enlund et al. unpublished data and ref. 51 and 52). An identical *CRTC1-MAML2* fusion has also been found in Warthin's tumors (**Fig. 1B**) and in clear cell hidradenomas of the skin,[45,53,54] indicating that the fusion is etiologically linked to benign and low-grade malignant, histogenetically related tumor types originating from diverse exocrine glands.

Much to our surprise we recently identified a second gene fusion in cutaneous hidradenomas and MEC. The fusion consists of the amino-terminal domain of EWSR1 and the DNA-binding, C-terminal domain of the transcription factor POU5F1 (**Fig. 1B**).[33] POU5F1 is expressed during early development to maintain the pluripotent status of embryonic stem and germ cells. Interestingly, the morphology of the *EWSR1-POU5F1* positive tumors were more immature compared to the *CRTC1-MAML2* positive tumors, raising the question of whether gene fusion could be a mechanism of POU5F1 reactivation leading to a more undifferentiated, stem cell-like phenotype in these tumors.

Recently, a hitherto unknown type of salivary gland tumor, mammary analogue secretory carcinoma of salivary glands, was described.[55] These tumors show strong histomorphologic and immunohistochemical resemblance to secretory carcinoma of the breast. In addition, both tumor types show an identical *ETV6-NTRK3* gene fusion (**Fig. 1C**), thus further emphasizing the histogenetic similarities between breast and salivary glands.

Finally, a recent study by Persson and co-workers identified a novel mechanism of activation of the *MYB* oncogene involving gene fusion in adenoid cystic carcinomas of the breast and head and neck.[34] In this fusion, a major part of *MYB* is linked to the last coding exon(s) of the transcription factor gene *NFIB* (**Fig. 1D**). The fusion results in overexpression of MYB-NFIB transcripts and protein as well as to transcriptional activation of critical *MYB* target genes. This study, together with previous and recent data showing rearrangements or copy number alterations of *MYB* in leukemias and certain solid tumors, will be the main focus of this review.

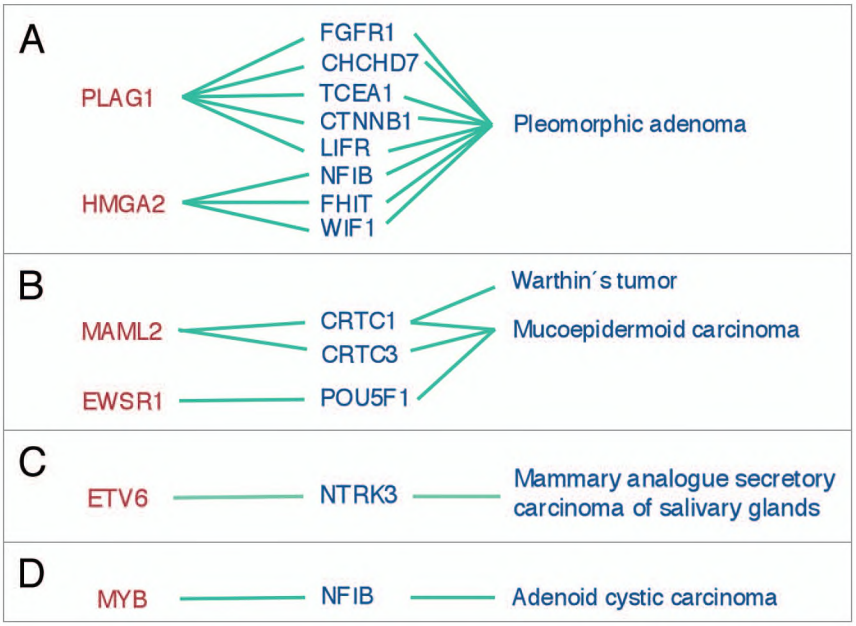


Figure 1. Gene fusions in salivary gland tumors. (A) *PLAG1*- and *HMGA2*-fusions in pleomorphic adenomas. (B) *MAML2*- and *POU5F1*-fusions in mucoepidermoid carcinomas and Warthin's tumors (metaplastic variant). (C) *ETV6-NTRK3* fusions in mammary analogue secretory carcinoma of salivary glands and (D) the recently identified novel *MYB-NFIB* fusion in adenoid cystic carcinoma.

© 2010 Landes Bioscience
Do not distribute

Identification of a Novel Mechanism of Activation of *MYB* Involving Gene Fusion

The *c-Myb* oncogene was first identified almost 30 years ago as the cellular homologue of the transforming *v-Myb* gene of two avian retroviruses that induce leukemia, avian myeloblastosis virus (AMV) and E26 leukemia virus.[56-59] The *v-myb* oncogenes of AMV and E26 encode N- and C-terminally truncated myb proteins. The murine *Myb* locus is also a common site of retroviral insertional mutagenesis.[60] MYB belongs to a family of transcription factors that include the closely related family members MYBL1 (a.k.a. AMYB) and MYBL2 (a.k.a. BMYB). MYB proteins function as transcriptional regulators and contain three functional key domains, an N-terminal DNA-binding domain comprised of three tandem 50 amino acid myb repeats that specifically bind to the sequence PyAACG/TG, a centrally located transcription activation domain and a C-terminal negative regulatory domain (NRD) involved in transcriptional repression (reviewed in refs. 61 and 62). The latter contains a leucine zipper-like motif and an EVES-motif that can modulate the activity of MYB. Disruption of the leucine zipper-like motif enhances the transforming activity of Myb and the EVES-motif has been shown to regulate the activity of Myb by inter- and intramolecular interactions.[63] Myb may also be sumoylated at two sites within the NRD leading to a reduction in Myb activity.[64]

The wild-type MYB-protein has a half-life of about 30 minutes and is post-translationally modified by phosphorylation, acetylation, sumoylation and ubiquitylation. These modifications may affect protein levels, DNA-binding and/or the transactivation capacity of MYB. MYB plays an important role in the control of cell proliferation, apoptosis and differentiation of in particular hematopoietic progenitor cells (reviewed in refs. 61 and 62). Loss of Myb function in mice results in embryonic lethality due to failure of fetal hepatic hematopoiesis and conditional *Myb* knockout in adult hematopoietic stem cells was recently shown to result in loss of self-renewal due to impaired

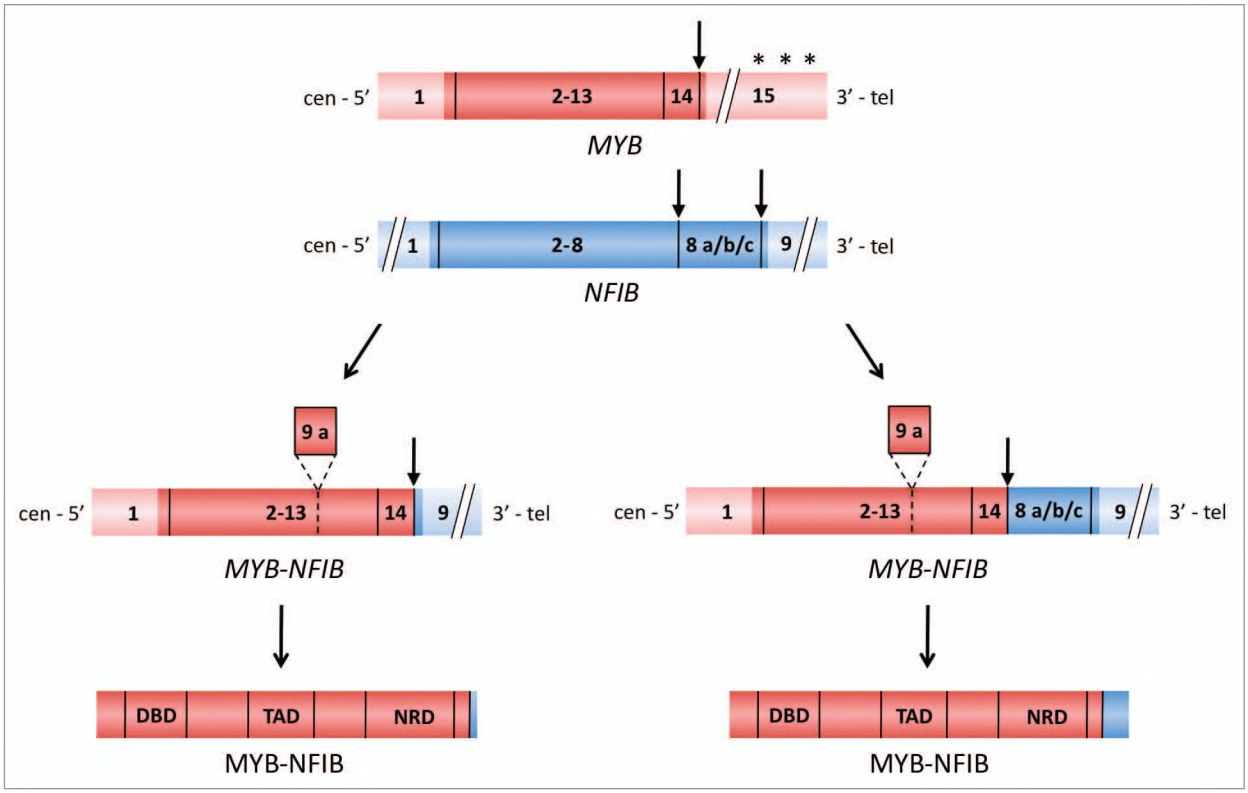


Figure 2. Schematic illustration of the *MYB* and *NFIB* genes and of two *MYB-NFIB* fusion variants (coding exons are shown i darker red and blue colors) and the resulting fusion proteins. Translocation breakpoints are shown by vertical arrows and miRNA binding sites for miR-15a/16 and miR-150 in the 3'-UTR of *MYB* are indicated by asteriks. The alternatively spliced *MYB* exon 9a and *NFIB* exons 8a, 8b and 8c are indicated. DBD, DNA binding domain; TAD, transactivation domain; NRD, negative regulatory domain.

proliferation and accelerated differentiation.[65,66] More recent work has also demonstrated that MYB is important during colon development (reviewed in ref. 62). MYB is highly expressed in immature, proliferating epithelial, endothelial and hematopoietic cells and is downregulated as cells become more differentiated. High expression levels of MYB have also been found in pancreatic, colon and breast tumors as well as in most leukemias and lymphomas (reviewed in ref. 62). Overexpression of *MYB* in colorectal cancer has been attributed to frequent mutations in attenuation sequences in the first intron that are known to regulate the transcription of MYB. In breast cancer, ERα (estrogen receptor-α) relieves the attenuation leading to increased expression of MYB, a characteristic feature of most ERα$^+$ breast cancers.

However, it was not until recently that clear evidence was presented showing that *MYB* actually is an oncogene rearranged in human cancer.[34,60,67] The most compelling evidence in support of this observation derives from studies of a recurrent t(6;9)(q22-23;p23-24) translocation in ACC.[34] This translocation was originally described in 1986 and was subsequently shown to be a primary and tumor-type specific aberration in ACC.[68,69] The t(6;9) translocation is found in approximately one third of karyotypically abnormal ACCs. Positional cloning of the 6q22-23 and 9p23-24 breakpoint regions revealed a fusion between the two transcription factor genes *MYB* and *NFIB*.[34] Due to alternative splicing and variable breakpoints in *MYB* and *NFIB*, at least 11 fusion transcript variants have been identified. Fusions consisting of *MYB* exon 14 linked to *NFIB* exons 8c and/or 9 predominate (**Fig. 2**), suggesting that most breakpoints occur in *MYB* intron 14 and in *NFIB* intron 8. The majority of transcripts contained one or more of the alternatively spliced *NFIB* exons 8a, 8a alternative, 8b, and/or 8c and MYB exon 9a. Preliminary studies of these transcript variants, although admittedly still limited, do not indicate that they have prognostic significance (unpublished data). However, since the fusion was found in a high frequency of ACCs, irrespective of whether they were derived from the breast, salivary glands, lacrimal glands or ceruminal glands of the ear, but not in any of 25 non-ACC tumor samples, we believe that the *MYB-NFIB* fusion is an important new diagnostic biomarker for this tumor entity. These findings unequivocally identify gene fusion as a novel mechanism for activation of *MYB* in human cancer and add to the evidence that *MYB* is an important human oncogene.

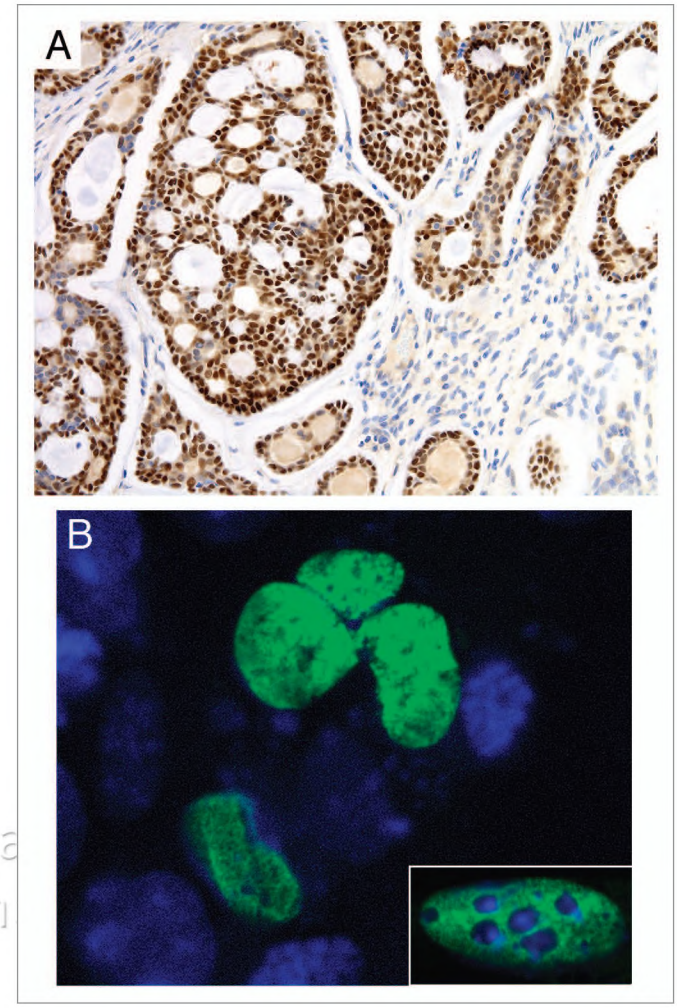
Molecular Consequences of the *MYB-NFIB* Gene Fusion

The expression of wild-type *MYB* is low in normal adult salivary gland and breast tissues.[34] In contrast, the *MYB-NFIB* fusion is highly expressed in translocation-positive tumors; the expression of the 5'-part

of *MYB* is significantly higher than that of the 3'-part, consistent with disruption of the 3'-part of the gene as a consequence of fusion to *NFIB*. This observation indicates that the increased *MYB* expression is not due to expression of the non-rearranged wild-type *MYB* allele. The MYB-NFIB protein is also highly expressed in fusion-positive ACCs (**Fig. 3A**). The immunoreactivity was, however, somewhat variable between tumors expressing similar levels of fusion transcript. The reason for this is unclear but could for example be due to heterogeneous expression of the fusion protein in different tumor cell populations or to fixation artifacts of the tissue specimens. Transient transfection of CHEF/18,[70] and NIH-3T3 cells with a GFP-tagged *MYB-NFIB* construct confirmed the nuclear localization of the fusion oncoprotein. A diffuse nuclear staining was observed in both cell types (**Fig. 3B**).

The predicted MYB-NFIB fusion protein retains the DNA-binding and transactivation domains of wild-type MYB and is therefore expected to activate MYB target genes. Indeed, 14 of 16 confirmed *MYB* targets were overexpressed in fusion-positive tumors relative to normal salivary gland tissue (**Fig. 4**).[34] These included genes associated with cell cycle control (*CCNB1*, *CDC2* and *MAD1L1*), apoptosis (*API5*, *BCL2*, *BIRC3*, *HSPA8* and *SET*), cell growth and angiogenesis (*MYC*, *KIT*, *VEGFA*, *FGF2*, *CD53*) and cell adhesion (*CD34*). Several of these genes have previously also been reported to be overexpressed in ACC (reviewed in ref. 34), thus further emphasizing their potential role in the pathogenesis of ACC. Continued studies of these and other transcriptional targets of the *MYB-NFIB* fusion will provide important new insights into their potential role as new diagnostic biomarkers and therapeutic targets for ACC.

The molecular mechanism by which *MYB* is activated by the t(6;9) translocation is still obscure. Since the minimal common part of *MYB* lost due to gene fusion with *NFIB* is exon 15, including the 3'-UTR, it has been proposed that *MYB* may be deregulated as a result of loss of binding sites for negatively regulating miRNAs.[34]



©2010 La
Do n

Figure 3. MYB-NFIB is a nuclear protein. (A) Immunostaining of the MYB-NFIB fusion protein in a translocation-positive ACC. Note the predominant nuclear staining of tumor cells whereas stromal cells are negative. (B) Chinese hamster embryo fibroblasts (CHEF/18 cells) and NIH-3T3 cells (inset) transfected with a *MYB-NFIB-GFP* expression construct. Nuclei are counterstained in blue with 4′,6′-diamidino-2′-phenylindole dihydrochloride (DAPI). Fluorescent cells were fixed on glass slides and imaged using a Zeiss LSM510 META confocal microscope system.

Is the *MYB*-fusion Another Example of Disruption of miRNA-Directed Repression of an Oncogene Activated by Chromosomal Translocation?

As previously indicated, *MYB* expression is tightly regulated by attenuation sequences located in the first intron of the gene and by a leucine-rich NRD in the C-terminus (see above). Mutation of the attenuation sequences or truncation/disruption of the NRD correlate with elevated expression levels of *MYB* in both human and experimentally induced neoplasms. However, recent studies have also indicated that MYB is negatively regulated by several miRNAs. The perhaps most compelling evidence of the latter mechanism derives from studies of miR-150, a miRNA selectively expressed in mature, resting B and T cells, but not in their progenitors. miR-150 directly regulates MYB expression in vivo through two conserved target sites in the 3'-UTR of *MYB* mRNA, and recent studies have shown that Myb is highly expressed during B-cell activation in *Mir150*$^{-/-}$ knockout mice and conversely that Myb expression is significantly reduced in mice overexpressing miR-150.[71] These findings have subsequently been confirmed by others showing that miR-150 indeed is an important regulator of MYB expression during

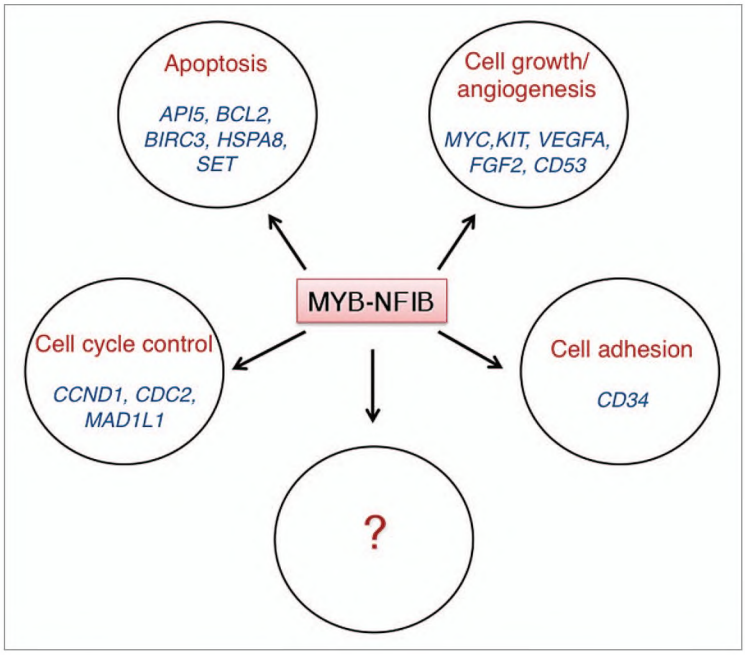


Figure 4. Confirmed MYB downstream target genes that are overexpressed in fusion-positive ACCs relative to normal salivary gland tissue.

differentiation.[72-75] In addition to miR-150, there are at least three other miRNAs, miR-15a, miR-16 and miR-34a, with target sites in the 3'-UTR of *MYB* that have been shown to negatively regulate *MYB* expression in vitro.[76-78] Thus, several lines of evidence point to an intricate miRNA-mediated regulation of *MYB* expression during development and differentiation.

Since the minimal common part of *MYB* lost due to gene fusion with *NFIB* is exon 15 (encoding the last 38 amino acids of the MYB protein), including the 3'-UTR, it has been proposed that *MYB* may be deregulated as a result of loss of binding sites for negatively regulating miRNAs in the 3'-UTR.[34] This hypothesis is supported by the observation that the most common fusion types do not disrupt the C-terminal NRD of MYB encoded by exons 10–13.[61,79-81] Forced overexpression of miR-150, miR-15a and miR-16 in short-term cultured fusion-positive ACC cells as well as in a T-cell acute lymphoblastic leukemia (T-ALL) cell line with MYB activation due to genomic duplication[67] resulted in a 30% downregulation of *MYB* mRNA in T-ALL cells after 45 hrs, whereas transfection of primary ACC cells did not significantly alter the expression of *MYB*. The reduction in *MYB* mRNA in the control T-ALL cells is comparable to previously published data.[73] The miRNAs miR-150 and miR-15a/16 are also expressed in fusion-positive ACCs as well as in normal salivary gland and breast tissues.[34] Taken together, these findings support the hypothesis that deletion of miRNA target sites in the 3'-UTR of *MYB* through gene fusion may disrupt the repression of MYB by miRNAs. Whether this is the major mechanism for activation of *MYB* in ACC or if also sequences in the 3'-UTR of *NFIB* (exon 9 only encodes the last five amino acids of the NFIB protein) and/or infrequent disruption of the MYB NRD may contribute to the deregulation of *MYB* remains to be elucidated.

In this respect *MYB* is reminiscent of *HMGA2*, another oncogene frequently activated by chromosomal translocations. Recent studies have shown that deregulation of *HMGA2* by loss of negatively regulating Let-7 miRNA target sites in the 3'-UTR due to gene fusion, is an important pathogenetic mechanism in several tumor types, including pleomorphic salivary gland adenomas.[9,41-43] Interestingly, in a subset of the latter cases the activation is due to fusions of the last five amino acids of the NFIB protein to the DNA-binding domains of HMGA2,[9,82] that is the same part of NFIB that is fused to MYB in ACC. Taken together available data, although admittedly still limited, suggest that MYB-NFIB may be another example of disruption of miRNA-directed repression of an oncogene activated by chromosomal translocations. However, additional studies are necessary to clarify the molecular mechanism underlying the activation of *MYB* by the t(6;9) and the potential contribution of *NFIB*.

Copy Number Alterations and Genomic Rearrangements of *MYB* in T-ALL and Epithelial Cancers

As predicted from the early studies demonstrating that Myb is leukemogenic in several animal species, it has now become clear that *MYB* is also rearranged in human leukemias. High-resolution comparative genomic hybridization array studies have revealed that a subset of T-ALL show selective amplification and overexpression of *MYB* (*MYB*^{dup}).[60,67] Interestingly, knockdown of *MYB* expression in T-ALL cell lines induces T-cell differentiation but has only limited effects on viability and cell proliferation.[67] However, γ-secretase inhibitors were shown to synergize with *MYB* knockdown to inhibit the growth of *MYB*^{dup} T-ALL cells in cases with concomitant *NOTCH1* mutations, suggesting that MYB indeed may be an interesting target for therapy in T-ALL. Studies on the mechanism by which the *MYB* duplications occur in T-ALL indicate that they are mediated somatically by homologous recombination between Alu-elements flanking the *MYB* locus.[83] In all cases analyzed, *MYB* was tandemly duplicated on one chromosome 6. The *Myb* locus is also a frequent insertion site in retrovirally-induced leukemias in mice; there are multiple known integration sites located both up- and downstream of *Myb* (reviewed in ref. 60). Recent studies have also identified a novel subgroup of T-ALL with t(6;7)(q23;q34) translocations that target *MYB*.[60] In these cases *MYB* and *TCRB* (T-cell receptor beta) are juxtaposed on the derivative chromosome 6, leading to activation of *MYB* expression (Fig. 5). The translocation breakpoints in 6q23 maps to two clusters located up to more than 50 kilobases telomeric of *MYB*. The *TCRB-MYB* translocation, which does not result in a true fusion gene,

©2010 Landes Bioscience. Do not distribute

defines a new subgroup of T-ALL associated with very young age and a proliferation/mitosis signature.

There are also examples of solid tumors in which *MYB* has been identified as a target for gene amplification. Thus, about one third of *BRCA1* mutated hereditary breast cancers show amplification of *MYB* and a corresponding overexpression of MYB mRNA and protein.[84] In contrast, *MYB* amplification is only found in 2% of sporadic breast cancers. Furthermore, selective *MYB* amplification has been found in approximately 10% of pancreatic cancers[85] as well as in two colorectal cancer cell lines[86] and two glioblastoma cell lines.[87] The above-mentioned studies, together with our recent findings of *MYB-NFIB* gene fusions in ACC, provide ample evidence that *MYB* indeed is an important human oncogene that is rearranged in a variety of human neoplasias originating from multiple cell lineages. Large-scale sequencing is currently changing our perceptions of the cancer genome, and by using high-resolution copy number arrays in combination with paired-end transcriptome sequencing strategies,[88,89] we expect that additional *MYB* gene rearrangements, including gene fusions, will be found also in other tumor types.

Perspectives: Towards Development of New Diagnostic and Therapeutic Strategies Based on the *MYB-NFIB* Fusion Oncogene

Previous studies of ACC has been seriously hampered by a number of factors, including a limited knowledge of the cellular and molecular biology of ACC as well as of the pathogenetic mechanisms leading to tumorigenesis, a lack of relevant in vitro and animal models to study disease pathogenesis and treatment, and a paucity of critical targets for development of new therapy. In particular, the fact that six commonly used ACC cell lines recently were shown to be cross-contaminated or misidentified has prompted a re-evaluation of published data regarding signaling pathways and new targets for therapy in ACC.[90] The recent discovery of the *MYB-NFIB* gene fusion provides important clues to the molecular pathogenesis of this highly malignant tumor and opens up new avenues for improved diagnosis and identification of novel targets for pharmaceutical intervention.

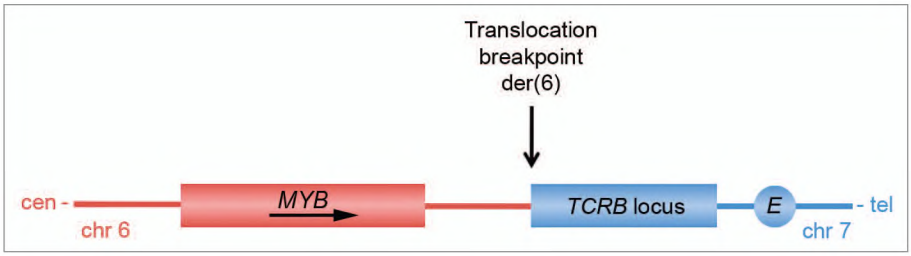


Figure 5. Schematic illustration of the translocation breakpoint region on the der(6) marker chromosome generated by the t(6;7)(q23;q34) translocation in T-ALL. Note that this translocation, unlike the t(6;9) in ACC, does not result in a *MYB* gene fusion. cen, centromere; tel, telomere. Adapted from reference 60.

The finding of the MYB-NFIB fusion in a high frequency of ACCs suggests that it may be used as a new diagnostic biomarker. We have recently developed a robust RT-PCR screening method for the fusion in formalin-fixed paraffin embedded tumor material (unpublished data) which should be particularly useful in the differential diagnosis of ACC. FISH using *MYB-NFIB* specific probes and immunohistochemistry using antibodies to the MYB or MYB-NFIB proteins will also be powerful adjunctive diagnostic tools. Efforts are also underway to develop noninvasive methods for detection of *MYB-NFIB* fusion transcripts in saliva from patients with primary salivary gland ACC. Similar studies of *ERG* gene fusions in urine samples from patients with prostate cancer has recently proved to be useful for noninvasive detection of prostate cancer.[91,92]

ACC is a slow-growing tumor with an often protracted clinical course. The poor long-term prognosis for these patients is mainly due to local recurrences and late onset of distant metastases.[93] To date, there is no effective systemic therapy available that inhibits the progression of the disease and improves the survival of ACC patients. Therefore, new therapeutic approaches are needed. As previously indicated, the *MYB-NFIB* fusion is a promising target for development of new therapeutic strategies for patients with this disease.[34] However, targeting transcription factors has proven to be notoriously difficult and accordingly there are few options available to directly target MYB. One such possibility was recently described by Williams and co-workers[94] who used a DNA vaccine encoding a fusion protein in which MYB was flanked by two tetanus toxin peptides. This vaccine was shown to significantly suppress tumor growth of an aggressive colon cancer syngenic transplant. It would be interesting to see whether such a vaccine also has similar effects against fusion-positive ACC xenografts in mice. In addition, clinical trials using antisense *MYB* oligodeoxinucleotides (ODN) have been conducted in patients with chronic myelogenous leukemia and other refractory leukemias.[95]

An alternative therapeutic approach for fusion-positive ACCs would be to target the genes that are regulated by the MYB-NFIB fusion. Studies are now in progress to identify new transcriptional targets and to verify those that were recently suggested (**Fig. 4**). Among the latter are several that have recently been subjected to pharmaceutical intervention. For example, VEGFA may be inhibited by bevacizumab, a humanized VEGF-antibody[96] as well as by several VEGFR inhibitors,[97] and BCL2 is inhibited by the small molecules ABT-737 and the related orally active ABT-263.[98] Moreover, the tyrosine kinase inhibitor (TKI) PD173074 inhibits FGF2 mediated proliferation through interaction with FGFR-1 and FGFR-2,[99,100] and the pan histone deacetylase inhibitor LBH589 (panobinostat) has been shown to downregulate both *MYC* and *MADIL1*[101] whereas *BIRC3* (*CIAP2*) and *BCL2* may be downregulated by for example fucoxantin and fucoxantinol.[102] KIT, which is a tyrosine kinase receptor for stem cell factor, is effectively inhibited by the TKI

© 2010 Landes Bioscience
Do not distribute

imatinib mesylate. However, despite the fact that KIT is highly overexpressed both at the RNA and protein levels in most cases of ACC, clinical studies have shown that imatinib has no major effects on advanced ACC of the head and neck.[103,104] With the exception of imatinib, the above-mentioned drugs and drugs with similar target specificities are interesting examples of potential MYB target gene inhibitors that deserve to be tested as single agents or in combination in in vitro and/or xenograft models to evaluate their efficacy in the treatment of ACC. Such efforts will hopefully lead to the emergence of potent therapies that will improve the survival of patients affected by this disease.

Acknowledgements

This work was supported by the Swedish Cancer Society and the IngaBritt and Arne Lundberg Research Foundation. We thank Ulric Pedersen for help preparing the illustrations. The authors have declared that no competing interests exist.

References

1. Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. Nat Rev Cancer 2007; 7:233-45.
2. Mitelman F, Johansson B, Mertens F, eds. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer: http://cgap.nci.nih.gov/Chromosomes/Mitelman 2010.
3. Asp J, Persson F, Kost-Alimova M, Stenman G. CHCHD7-PLAG1 and TCEA1-PLAG1 gene fusions resulting from cryptic, intrachromosomal 8q rearrangements in pleomorphic salivary gland adenomas. Genes Chromosomes Cancer 2006; 45:820-8.
4. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 2007; 448:595-9.
5. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007; 448:561-6.
6. Persson F, Winnes M, Andren Y, Wedell B, Dahlenfors R, Asp J, et al. High-resolution array CGH analysis of salivary gland tumors reveals fusion and amplification of the FGFR1 and PLAG1 genes in ring chromosomes. Oncogene 2008; 27:3072-80.
7. Persson F, Andren Y, Winnes M, Wedell B, Nordkvist A, Gudnadottir G, et al. High-resolution genomic profiling of adenomas and carcinomas of the salivary glands reveals amplification, rearrangement and fusion of HMGA2. Genes Chromosomes Cancer 2009; 48:69-82.
8. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 2007; 131:1190-203.
9. Stenman G. Fusion oncogenes and tumor type specificity—insights from salivary gland tumors. Semin Cancer Biol 2005; 15:224-35.
10. Edwards PA. Fusion genes and chromosome translocations in the common epithelial cancers. J Pathol 2010; 220:244-54.
11. Jhiang SM, Sagartz JE, Tong Q, Parker-Thornburg J, Capen CC, Cho JY, et al. Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. Endocrinology 1996; 137:375-8.
12. Santoro M, Chiappetta G, Cerrato A, Salvatore D, Zhang L, Manzo G, et al. Development of thyroid papillary carcinomas secondary to tissue-specific expression of the RET/PTC1 oncogene in transgenic mice. Oncogene 1996; 12:1821-6.
13. Perez-Losada J, Pintado B, Gutierrez-Adan A, Flores T, Banares-Gonzalez B, del Campo JC, et al. The chimeric FUS/TLS-CHOP fusion protein specifically induces liposarcomas in transgenic mice. Oncogene 2000; 19:2413-22.
14. Haldar M, Hancock JD, Coffin CM, Lessnick SL, Capecchi MR. A conditional mouse model of synovial sarcoma: insights into a myogenic origin. Cancer Cell 2007; 11:375-88.
15. Keller C, Arenkiel BR, Coffin CM, El-Bardeesy N, DePinho RA, Capecchi MR. Alveolar rhabdomyosarcomas in conditional Pax3:Fkhr mice: Cooperativity of Ink4a/ARF and Trp53 loss of function. Genes Dev 2004; 18:2614-26.
16. Li Z, Tognon CE, Godinho FJ, Yasaitis L, Hock H, Herschkowitz JI, et al. ETV6-NTRK3 fusion oncogene initiates breast cancer from committed mammary progenitors via activation of AP1 complex. Cancer Cell 2007; 12:542-58.
17. Soda M, Takada S, Takeuchi K, Choi YL, Enomoto M, Ueno T, et al. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci USA 2008; 105:19893-7.
18. Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. Nat Genet 2009; 41:619-24.
19. King JC, Xu J, Wongvipat J, Hieronymus H, Carver BS, Leung DH, et al. Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. Nat Genet 2009; 41:524-6.
20. Helman LJ, Meltzer P. Mechanisms of sarcoma development. Nat Rev Cancer 2003; 3:685-94.
21. Dauphinot L, De Oliveira C, Melot T, Sevenet N, Thomas V, Weissman BE, et al. Analysis of the expression of cell cycle regulators in Ewing cell lines: EWS-FLI-1 modulates $p57^{KIP2}$ and c-Myc expression. Oncogene 2001; 20:3258-65.
22. Matsumoto Y, Tanaka K, Nakatani F, Matsunobu T, Matsuda S, Iwamoto Y. Downregulation and forced expression of EWS-Fli1 fusion gene results in changes in the expression of G(1)regulatory genes. Br J Cancer 2001; 84:768-75.
23. Li X, Tanaka K, Nakatani F, Matsunobu T, Sakimura R, Hanada M, et al. Transactivation of cyclin E gene by EWS-Fli1 and antitumor effects of cyclin dependent kinase inhibitor on Ewing's family tumor cells. Int J Cancer 2005; 116:385-94.
24. Nakatani F, Tanaka K, Sakimura R, Matsumoto Y, Matsunobu T, Li X, et al. Identification of $p21^{WAF1/CIP1}$ as a direct target of EWS-Fli1 oncogenic fusion protein. J Biol Chem 2003; 278:15105-15.
25. Sanchez G, Bittencourt D, Laud K, Barbier J, Delattre O, Auboeuf D, et al. Alteration of cyclin D1 transcript elongation by a mutated transcription factor upregulates the oncogenic D1b splice isoform in cancer. Proc Natl Acad Sci USA 2008; 105:6004-9.
26. Tognon C, Garnett M, Kenward E, Kay R, Morrison K, Sorensen PH. The chimeric protein tyrosine kinase ETV6-NTRK3 requires both Ras-Erk1/2 and PI3-kinase-Akt signaling for fibroblast transformation. Cancer Res 2001; 61:8909-16.
27. Xie Y, Skytting B, Nilsson G, Gasbarri A, Haslam K, Bartolazzi A, et al. SYT-SSX is critical for cyclin D1 expression in synovial sarcoma cells: A gain of function of the t(X;18)(p11.2;q11.2) translocation. Cancer Res 2002; 62:3861-7.
28. Cassinelli G, Favini E, Degl'Innocenti D, Salvi A, De Petro G, Pierotti MA, et al. RET/PTC1-driven neoplastic transformation and proinvasive phenotype of human thyrocytes involve Met induction and beta-catenin nuclear translocation. Neoplasia 2009; 11:10-21.
29. Zhang L, Wang C. PAX3-FKHR transformation increases 26 S proteasome-dependent degradation of $p27^{Kip1}$, a potential role for elevated Skp2 expression. J Biol Chem 2003; 278:27-36.
30. Bento C, Andersson MK, Aman P. DDIT3/CHOP and the sarcoma fusion oncoprotein FUS-DDIT3/TLS-CHOP bind cyclin-dependent kinase 2. BMC Cell Biol 2009; 10:89.
31. Shah JP, Ihde JK. Salivary gland tumors. Curr Probl Surg 1990; 27:775-883.
32. Barnes L, Eveson JW, Reichart P, Sidransky D, eds. World health organization classification of tumours. Pathology and genetics of head and neck tumours. Lyon: IARC Press 2005; 209-74.
33. Moller E, Stenman G, Mandahl N, Hamberg H, Molne L, van den Oord JJ, et al. POU5F1, encoding a key regulator of stem cell pluripotency, is fused to EWSR1 in hidradenoma of the skin and mucoepidermoid carcinoma of the salivary glands. J Pathol 2008; 215:78-86.
34. Persson M, Andren Y, Mark J, Horlings HM, Persson F, Stenman G. Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. Proc Natl Acad Sci USA 2009; 106:18740-4.
35. Kas K, Voz ML, Roijer E, Astrom AK, Meyen E, Stenman G, et al. Promoter swapping between the genes for a novel zinc finger protein and beta-catenin in pleiomorphic adenomas with t(3;8)(p21;q12) translocations. Nat Genet 1997; 15:170-4.
36. Voz ML, Mathys J, Hensen K, Pendeville H, Van Valckenborgh I, Van Huffel C, et al. Microarray screening for target genes of the proto-oncogene PLAG1. Oncogene 2004; 23:179-91.
37. Landrette SF, Kuo YH, Hensen K, Barjesteh van Waalwijk van Doorn-Khosrovani S, Perrat PN, Van de Ven WJ, et al. Plag1 and Plagl2 are oncogenes that induce acute myeloid leukemia in cooperation with Cbfb-MYH11. Blood 2005; 105:2900-7.
38. Ashar HR, Chouinard RA Jr, Dokur M, Chada K. In vivo modulation of HMGA2 expression. Biochim Biophys Acta 2010; 1799:55-61.
39. Tessari MA, Gostissa M, Altamura S, Sgarra R, Rustighi A, Salvagno C, et al. Transcriptional activation of the cyclin A gene by the architectural transcription factor HMGA2. Mol Cell Biol 2003; 23:9104-16.
40. De Martino I, Visone R, Wierinckx A, Palmieri D, Ferraro A, Cappabianca P, et al. HMGA proteins upregulate CCNB2 gene in mouse and human pituitary adenomas. Cancer Res 2009; 69:1844-50.
41. Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev 2007; 21:1025-30.
42. Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science 2007; 315:1576-9.
43. Klemke M, Meyer A, Hashemi Nezhad M, Belge G, Bartnitzke S, Bullerdiek J. Loss of let-7 binding sites resulting from truncations of the 3' untranslated region of HMGA2 mRNA in uterine leiomyomas. Cancer Genet Cytogenet 2010; 196:119-23.
44. Tonon G, Modi S, Wu L, Kubo A, Coxon AB, Komiya T, et al. t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. Nat Genet 2003; 33:208-13.
45. Enlund F, Behboudi A, Andren Y, Oberg C, Lendahl U, Mark J, et al. Altered Notch signaling resulting from expression of a WAMTP1-MAML2 gene fusion in mucoepidermoid carcinomas and benign Warthin's tumors. Exp Cell Res 2004; 292:21-8.

©2010 Landes Bioscience. Do not distribute.

46. Behboudi A, Enlund F, Winnes M, Andren Y, Nordkvist A, Leivo I, et al. Molecular classification of mucoepidermoid carcinomas-prognostic significance of the MECT1-MAML2 fusion oncogene. Genes Chromosomes Cancer 2006; 45:470-81.
47. Lin SE, Oyama T, Nagase T, Harigaya K, Kitagawa M. Identification of new human mastermind proteins defines a family that consists of positive regulators for notch signaling. J Biol Chem 2002; 277:50612-20.
48. Wu L, Sun T, Kobayashi K, Gao P, Griffin JD. Identification of a family of mastermind-like transcriptional coactivators for mammalian notch receptors. Mol Cell Biol 2002; 22:7688-700.
49. Conkright MD, Canettieri G, Screaton R, Guzman E, Miraglia L, Hogenesch JB, et al. TORCs: Transducers of regulated CREB activity. Mol Cell 2003; 12:413-23.
50. Iourgenko V, Zhang W, Mickanin C, Daly I, Jiang C, Hexham JM, et al. Identification of a family of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. Proc Natl Acad Sci USA 2003; 100:12147-52.
51. Coxon A, Rozenblum E, Park YS, Joshi N, Tsurutani J, Dennis PA, et al. Mect1-Maml2 fusion oncogene linked to the aberrant activation of cyclic AMP/CREB regulated genes. Cancer Res 2005; 65:7137-44.
52. Wu L, Liu J, Gao P, Nakamura M, Cao Y, Shen H, et al. Transforming activity of MECT1-MAML2 fusion oncoprotein is mediated by constitutive CREB activation. EMBO J 2005; 24:2391-402.
53. Fehr A, Roser K, Belge G, Loning T, Bullerdiek J. A closer look at Warthin tumors and the t(11;19). Cancer Genet Cytogenet 2008; 180:135-9.
54. Winnes M, Molne L, Suurkula M, Andren Y, Persson F, Enlund F, et al. Frequent fusion of the CRTC1 and MAML2 genes in clear cell variants of cutaneous hidradenomas. Genes Chromosomes Cancer 2007; 46:559-63.
55. Skalova A, Vanecek T, Sima R, Laco J, Weinreb I, Perez-Ordonez B, et al. Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. Am J Surg Pathol 2010; 34:599-608.
56. Duesberg PH, Bister K, Moscovici C. Genetic structure of avian myeloblastosis virus, released from transformed myeloblasts as a defective virus particle. Proc Natl Acad Sci USA 1980; 77:5120-4.
57. Souza LM, Strommer JN, Hillyard RL, Komaromy MC, Baluda MA. Cellular sequences are present in the presumptive avian myeloblastosis virus genome. Proc Natl Acad Sci USA 1980; 77:5177-81.
58. Baluda MA, Reddy EP. Anatomy of an integrated avian myeloblastosis provirus: structure and function. Oncogene 1994; 9:2761-74.
59. Lipsick JS, Wang DM. Transformation by v-Myb. Oncogene 1999; 18:3047-55.
60. Clappier E, Cuccuini W, Kalota A, Crinquette A, Cayuela JM, Dik WA, et al. The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. Blood 2007; 110:1251-61.
61. Oh IH, Reddy EP. The myb gene family in cell growth, differentiation and apoptosis. Oncogene 1999; 18:3017-33.
62. Ramsay RG, Gonda TJ. MYB function in normal and cancer cells. Nat Rev Cancer 2008; 8:523-34.
63. Dash AB, Orrico FC, Ness SA. The EVES motif mediates both intermolecular and intramolecular regulation of c-Myb. Genes Dev 1996; 10:1858-69.
64. Molvaersmyr AK, Saether T, Gilfillan S, Lorenzo PI, Kvaloy H, Matre V, et al. A SUMO-regulated activation function controls synergy of c-Myb through a repressor-activator switch leading to differential p300 recruitment. Nucleic Acids Res 2010; 38:4970-84.
65. Mucenski ML, McLain K, Kier AB, Swerdlow SH, Schreiner CM, Miller TA, et al. A functional c-myb gene is required for normal murine fetal hepatic hematopoiesis. Cell 1991; 65:677-89.
66. Lieu YK, Reddy EP. Conditional c-myb knockout in adult hematopoietic stem cells leads to loss of self-renewal due to impaired proliferation and accelerated differentiation. Proc Natl Acad Sci USA 2009; 106:21689-94.
67. Lahortiga I, De Keersmaecker K, Van Vlierberghe P, Graux C, Cauwelier B, Lambert F, et al. Duplication of the MYB oncogene in T cell acute lymphoblastic leukemia. Nat Genet 2007; 39:593-5.
68. Stenman G, Sandros J, Dahlenfors R, Juberg-Ode M, Mark J. 6q- and loss of the Y chromosome—two common deviations in malignant human salivary gland tumors. Cancer Genet Cytogenet 1986; 22:283-93.
69. Nordkvist A, Mark J, Gustafsson H, Bang G, Stenman G. Non-random chromosome rearrangements in adenoid cystic carcinoma of the salivary glands. Genes Chromosomes Cancer 1994; 10:115-21.
70. Stenman G, Delorme EO, Lau CC, Sager R. Transfection with plasmid pSV2gptEJ induces chromosome rearrangements in CHEF cells. Proc Natl Acad Sci USA 1987; 84:184-8.
71. Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, Wang J, et al. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. Cell 2007; 131:146-59.
72. Barroga CF, Pham H, Kaushansky K. Thrombopoietin regulates c-Myb expression by modulating micro RNA 150 expression. Exp Hematol 2008; 36:1585-92.
73. Lin YC, Kuo MW, Yu J, Kuo HH, Lin RJ, Lo WL, et al. c-Myb is an evolutionary conserved miR-150 target and miR-150/c-Myb interaction is important for embryonic development. Mol Biol Evol 2008; 25:2189-98.
74. Tan LP, Wang M, Robertus JL, Schakel RN, Gibcus JH, Diepstra A, et al. miRNA profiling of B-cell subsets: specific miRNA profile for germinal center B cells with variation between centroblasts and centrocytes. Lab Invest 2009; 89:708-16.
75. Venugopal SK, Jiang J, Kim TH, Li Y, Wang SS, Torok NJ, et al. Liver fibrosis causes downregulation of miRNA-150 and miRNA-194 in hepatic stellate cells and their overexpression causes decreased stellate cell activation. Am J Physiol Gastrointest Liver Physiol 2009; 298:G101-6.
76. Chung EY, Dews M, Cozma D, Yu D, Wentzel EA, Chang TC, et al. c-Myb oncoprotein is an essential target of the dleu2 tumor suppressor microRNA cluster. Cancer Biol Ther 2008; 7:1758-64.
77. Navarro F, Gutman D, Meire E, Caceres M, Rigoutsos I, Bentwich Z, et al. miR-34a contributes to megakaryocytic differentiation of K562 cells independently of p53. Blood 2009; 114:2181-92.
78. Zhao H, Kalota A, Jin S, Gewirtz AM. The c-myb proto-oncogene and microRNA-15a comprise an active autoregulatory feedback loop in human hematopoietic cells. Blood 2009; 113:505-16.
79. Sakura H, Kanei-Ishii C, Nagase T, Nakagoshi H, Gonda TJ, Ishii S. Delineation of three functional domains of the transcriptional activator encoded by the c-myb protooncogene. Proc Natl Acad Sci USA 1989; 86:5758-62.
80. Dubendorff JW, Whittaker LJ, Eltman JT, Lipsick JS. Carboxy-terminal elements of c-Myb negatively regulate transcriptional activation in cis and in trans. Genes Dev 1992; 6:2524-35.
81. Press RD, Reddy EP, Ewert DL. Overexpression of C-terminally but not N-terminally truncated Myb induces fibrosarcomas: a novel nonhematopoietic target cell for the myb oncogene. Mol Cell Biol 1994; 14:2278-90.
82. Geurts JM, Schoenmakers EF, Roijer E, Astrom AK, Stenman G, van de Ven WJ. Identification of NFIB as recurrent translocation partner gene of HMGIC in pleomorphic adenomas. Oncogene 1998; 16:865-72.
83. O'Neil J, Tchinda J, Gutierrez A, Moreau L, Maser RS, Wong KK, et al. Alu elements mediate MYB gene tandem duplication in human T-ALL. J Exp Med 2007; 204:3059-66.
84. Kauraniemi P, Hedenfalk I, Persson K, Duggan DJ, Tanner M, Johannsson O, et al. MYB oncogene amplification in hereditary BRCA1 breast cancer. Cancer Res 2000; 60:5323-8.
85. Wallrapp C, Muller-Pillasch F, Solinas-Toldo S, Lichter P, Friess H, Buchler M, et al. Characterization of a high copy number amplification at 6q24 in pancreatic cancer identifies c-myb as a candidate oncogene. Cancer Res 1997; 57:3135-9.
86. Alitalo K, Winqvist R, Lin CC, de la Chapelle A, Schwab M, Bishop JM. Aberrant expression of an amplified c-myb oncogene in two cell lines from a colon carcinoma. Proc Natl Acad Sci USA 1984; 81:4534-8.
87. Welter C, Henn W, Theisinger B, Fischer H, Zang KD, Blin N. The cellular myb oncogene is amplified, rearranged and activated in human glioblastoma cell lines. Cancer Lett 1990; 52:57-62.
88. Maher CA, Kumar-Sinha C, Cao X, Kalyana-Sundaram S, Han B, Jing X, et al. Transcriptome sequencing to detect gene fusions in cancer. Nature 2009; 458:97-101.
89. Maher CA, Palanisamy N, Brenner JC, Cao X, Kalyana-Sundaram S, Luo S, et al. Chimeric transcript discovery by paired-end transcriptome sequencing. Proc Natl Acad Sci USA 2009; 106:12353-8.
90. Phuchareon J, Ohta Y, Woo JM, Eisele DW, Tetsu O. Genetic profiling reveals cross-contamination and misidentification of 6 adenoid cystic carcinoma cell lines: ACC2, ACC3, ACCM, ACCNS, ACCS and CAC2. PLoS One 2009; 4:6040.
91. Laxman B, Tomlins SA, Mehra R, Morris DS, Wang L, Helgeson BE, et al. Noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of men with prostate cancer. Neoplasia 2006; 8:885-8.
92. Rice KR, Chen Y, Ali A, Whitman EJ, Blase A, Ibrahim M, et al. Evaluation of the ETS-related gene mRNA in urine for the detection of prostate cancer. Clin Cancer Res 2010; 16:1572-6.
93. Spiro RH. Salivary neoplasms: overview of a 35-year experience with 2,807 patients. Head Neck Surg 1986; 8:177-84.
94. Williams BB, Wall M, Miao RY, Williams B, Bertoncello I, Kershaw MH, et al. Induction of T cell-mediated immunity using a c-Myb DNA vaccine in a mouse model of colon cancer. Cancer Immunol Immunother 2008; 57:1635-45.
95. Gewirtz AM. Myb targeted therapeutics for the treatment of human malignancies. Oncogene 1999; 18:3056-62.
96. Presta LG, Chen H, O'Connor SJ, Chisholm V, Meng YG, Krummen L, et al. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 1997; 57:4593-9.
97. Ivy SP, Wick JY, Kaufman BM. An overview of small-molecule inhibitors of VEGFR signaling. Nat Rev Clin Oncol 2009; 6:569-79.
98. Vogler M, Dinsdale D, Dyer MJ, Cohen GM. Bcl-2 inhibitors: small molecules with a big impact on cancer therapy. Cell Death Differ 2009; 16:360-7.

©Landes Bioscience. Do not distribute.

99. Bansal R, Magge S, Winkler S. Specific inhibitor of FGF receptor signaling: FGF-2-mediated effects on proliferation, differentiation and MAPK activation are inhibited by PD173074 in oligodendrocyte-lineage cells. J Neurosci Res 2003; 74:486-93.
100. Pardo OE, Latigo J, Jeffery RE, Nye E, Poulsom R, Spencer-Dene B, et al. The fibroblast growth factor receptor inhibitor PD173074 blocks small cell lung cancer growth in vitro and in vivo. Cancer Res 2009; 69:8645-51.
101. Prystowsky MB, Adomako A, Smith RV, Kawachi N, McKimpson W, Atadja P, et al. The histone deacetylase inhibitor LBH589 inhibits expression of mitotic genes causing G_2/M arrest and cell death in head and neck squamous cell carcinoma cell lines. J Pathol 2009; 218:467-77.
102. Ishikawa C, Tafuku S, Kadekaru T, Sawada S, Tomita M, Okudaira T, et al. Anti-adult T-cell leukemia effects of brown algae fucoxanthin and its deacetylated product, fucoxanthinol. Int J Cancer 2008; 123:2702-12.
103. Hotte SJ, Winquist EW, Lamont E, MacKenzie M, Vokes E, Chen EX, et al. Imatinib mesylate in patients with adenoid cystic cancers of the salivary glands expressing c-kit: a Princess Margaret Hospital phase II consortium study. J Clin Oncol 2005; 23:585-90.
104. Pfeffer MR, Talmi Y, Catane R, Symon Z, Yosepovitch A, Levitt M. A phase II study of Imatinib for advanced adenoid cystic carcinoma of head and neck salivary glands. Oral Oncol 2007; 43:33-6.

©2010 Landes Bioscience.
Do not distribute.