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Review

Transcription factors involved in development and progression of malignant melanoma

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Summary. Up to date many genes are known to be deregulated in tumor development and progression. Genes important in tumorigenesis belong to families such as proteases, kinases and receptors. However, an important family of proteins is rarely discussed: the mediators of transcriptional control, the transcription factors. Usually, changes in transcription factor expression or activity can lead to more than just one downstream modification, as transcription factors are higher, thinking in a hierarchical way of expression control. In this review we summarize the role of the transcription factors AP-1, AP-2alpha, CREB, CtBP, ETS-1, HMGB1, LEF/TCF/ß-catenin, MITF, NFκB, PAX3, SKI, Snail and STAT in carcinogenesis focusing on melanoma development and progression.

Key words: Malignant melanoma, Tumor development, Transcription factor, Metastasis, Transcriptional regulation

Introduction

Melanoma progression and tumor growth are regulated by a complex network of paracrine and autocrine positive and negative growth factors. Several groups have searched for transcription factors being involved in this process. The fundamental role of gene transcription and the recognition of transcription factors as important control elements of cell growth, differentiation, and programmed cell death (apoptosis) can lead to a new understanding of tumor development. Changes in transcriptional regulation represent an early event in tumorigenesis either controlled by the tumor microenvironment or by mutations or modifications in the cell itself. There are several publications focusing on changes in expression patterns of several molecules in malignant melanoma, but only recently have the causes for these modified expression patterns started to be accessed.

Details of all mentioned transcription factors including chromosome location, size, general function, DNA-binding and protein-binding domain, respectively, as well as target genes are summarized in tables 1 and 2.

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AP-1

The activation protein 1 (AP-1) family of transcription factors consists of Jun (c-Jun, JunD, JunB) and Fos (c-Fos, Fra-1, Fra-2, FosB). Each protein contains a bZIP (basic region-leucine zipper) region consisting of a basic DNA-binding domain and a leucine zipper domain. Members of the Fos and Jun family heterodimerize to form DNA-binding complexes and subsequently stimulate transcription of genes containing the AP-1 consensus DNA-binding site TGA(C/G)TCA (Nakabeppu et al., 1988).

The oncogene jun was determined as the putative transforming gene of avian sarcoma virus 17 with several homologs in vertebrate species (Jun comes from Japanese “ju-nana” meaning “seventeen”). The jun gene has no introns and is located on human chromosome 1p32-p31. The human fos oncogene is homologous to the Finkel-Biskis-Jinkins (FBJ) murine osteosarcoma virus oncogene. It consists of 4 exons spanning approximately 4 kb on human chromosome 14q24.3-q31.

Interestingly, specific AP-1 dimer combinations exhibit considerable differences in their transactivating efficiency. For example, c-Jun is more effective in activating transcription of AP-1-responsive genes than JunB. In addition, JunB represses c-Jun functions by forming inactive c-Jun/JunB heterodimers (Chiu et al., 1989; Deng and Karin, 1993). In keratinocytes, Fra-2 and c-Fos play opposing roles in regulating AP-1 activity depending on treatment of the cells with 12-O-tetradecanoyl phorbol-13-acetate (TPA), an agent that induces terminal differentiation of keratinocytes (Rutberg et al., 1997).

There is experimental proof that Fos may transform fibroblasts through alterations in DNA methylation and

in histone deacetylation by activating DNA 5-methylcytosine transferase. Recent studies using transgenic and knockout mice have established a role of Fos in regulating the differentiation and activity of specific bone-cell populations, both during normal development and in bone disease (reviewed by Grigoriadis et al., 1995).

Urabe and co-workers (1992) showed a higher expression of the Fos gene product in basal cell carcinoma and malignant melanoma with histological evidence of invasiveness of the tumor cells. Thus, they suppose there is a correlation between higher expression of the fos oncogene and the malignant progression of tumor cells, in particular the extent of invasiveness.

Sequence analysis using the computer program GCG (genetic computer group) FINDPATTERN identified multiple recognition sites for the AP-1 and C/EBP (CCAAT/enhancer binding protein) transcription factors in a specific melanoma differentiation-associated (mda) gene, mda-7 (also named IL-24). The mRNA expression of mda-7 was found to be elevated in actively proliferating normal human melanocytes compared to primary and metastatic human melanomas (Huang et al., 2001). Transfection assays and western blot analyses revealed that c-Jun and the C/EBP family member C/EBP-beta are physiologically relevant transcription factors whose expression levels correspond to mda-7 mRNA expression. In contrast, a dominant negative mutant of c-Jun, TAM67, does not possess promoter-enhancing ability. These results provide further mechanistic insights into the regulation of the mda-7 gene during induction of terminal cell differentiation in human melanoma cells (Madireddi et al., 2000).

Furthermore, in a Matrigel-assisted melanoma

Table 1. Compendium of relevant DNA-binding transcription factors of melanoma development and progression.

TRANSCRIPTION FACTOR	CHROMOSOME	SIZE (kDa)	FUNCTION	DNA-BINDING DOMAIN	TARGET GENES
AP1 (c-fos)	14q24.3-q31	55	oncogene	TGA C/G TCA (“TRE”)	p21
(c-jun)	1p32-p31	43	oncogene	TGA C/G TCA (“TRE”)	mda-7, Fas, MMPC-7
AP2alpha	6p24-p22.3	52	activator	GCCNNNGGC	c-kit, E-cadherin, p21/WAF1, HER2/neu, Bcl-2, FAS/APO-1, IGF-R-1, VEGF, PAR-1, MCAM/MUC18
CREB	2q32.3-q34	43	activator	TGANNTCA (“CRE”)	Somatostatin, Proenkephalin, Fos, MMP2, MCAM/MUC18
ETS-1	11q23.3	51	activator/repressor	GGA A/T	MMP1, MMP3, MMP9, uPA, ß3 Integrin
HMGB1	13q12	32	activator/repressor	GGCATTT (“HCR”) (Poser et al., 2003)	MIA
LEF-1	4q22	55-57	activator/repressor	AGATCAAAGGG	MMP7, Myc, APCDD1, SOX2, Cyclin D1, TCF-1, MITF
TCF-4	10q25.3	60			
MITF	3p14.3-p12.3	526 aa	activator	AGTCATGTG (“M-box”)	Trp1, Trp2, Tyr, Bcl-2
NFκB (p50)	4q23-q24	50	activator	GGGRNNYYCC	c-myc, bcl-2
(p65)	11q12-q13	65	activator	GGGRNNYYCC	c-myc, bcl-2
PAX3	2q35	215 aa	activator	TCGTCAC G/C CN T/C N A	MITF
SKI	1p36.3	84	repressor	GTCTAGAC (“SBE”)	Smad3
Snail	20q13.1	264 aa	repressor	CANNTG (“E-box”)	E-cadherin, Vimentin
STAT1 (α/ß)	2q32.2	91/84	activator/repressor	TTCNNNGAA (“SIE-1”)	p21/WAF1
STAT3 (α/ß)	17q21	92/83	activator/repressor	TTCNNNGAA (“SIE-1”)	p21/WAF1

Table 2. Overview of relevant transcription factors for melanoma development and progression acting via protein-protein interactions.

TRANSCRIPTION FACTOR	CHROMOSOME	SIZE (kDa)	FUNCTION	DNA-BINDING DOMAIN	PROTEIN-BINDING DOMAIN	INTERACTING PROTEINS
CtBP	4p16	48	Co-repressor	none	PXDLS	TCF-3, TCF-4, delta-EF1, Hairy, Kruppel, Knirps, Snail, Giant, Cyclin D1, Evi-1
ß-Catenin (CTNNB1)	3p22-p21.3	92	Co-activator	none	Domain of 59 aa on N-terminus of TCF	LEF/TCF; cadherin, catenin
SKI	1p36.3	84	repressor	none	“SAND” KDWK motif	Smad3

progression model (Boyden Chamber), mda-7 expression is downregulated in early vertical growth phase primary human melanoma cells selected for autonomous or enhanced tumor formation in nude mice. Treatment of human melanoma cells with recombinant interferon (IFN)-beta and the protein kinase C activator mezerein (MEZ) results in an irreversible loss in growth potential, suppression of tumorigenic properties and induction of terminal cell differentiation. All effects have been shown to be associated with elevated mda-7 expression (Huang et al., 2001).

Moreover, mda-7 is assumed to function as a negative regulator of melanoma progression, since transfection of mda-7 expression constructs into H0-1 and C8161 human melanoma cells reduces growth and inhibits colony formation. These results confirm that mda-7 has antiproliferative properties in human melanoma cells and in this context may contribute to terminal cell differentiation (Jiang et al., 1995).

AP-2alpha

Activating enhancer-binding protein 2 alpha (AP-2alpha) is a helix-turn-helix protein of 52 kDa that binds to the consensus DNA sequence GCCNNNGGC as a homodimer (Williams et al., 1988). Together with AP-2beta and AP-2gamma it is a member of a transcription factor family combining the three closely related and evolutionarily conserved sequence-specific DNA-binding proteins (reviewed by Hilger-Eversheim et al., 2000).

The human AP-2alpha gene was mapped to human chromosome 6p24 – p22.3 and consists of 7 exons distributed over 18 kb (Bauer et al., 1994). AP-2alpha was shown to be expressed in tissues of ectodermal origin and homozygous AP-2alpha-deficient mice revealed dramatic neural tube defects (Schorle et al., 1996). As the result of a subtractive cloning approach it was suggested that AP-2alpha might be required for cell proliferation by suppression of genes inducing terminal differentiation, apoptosis, and growth retardation (Pfisterer et al., 2002). Furthermore, c-kit, E-cadherin, p21/WAF1, HER2/neu, Bcl-2, FAS/APO-1, IGF-R-1, VEGF, and the thrombin receptor (PAR-1) are discussed as AP-2 target genes (Bar-Eli, 2001).

Recently, loss of AP-2alpha expression has been demonstrated for malignant melanoma (Huang et al., 1998; Jean et al., 1998a). The results of these studies indicated that expression of the cell adhesion molecule MCAM/MUC18 and of c-KIT, both strongly upregulated in malignant melanoma, are negatively regulated by AP-2. These results were supported by two immunohistochemical studies (Karjalainen et al., 1998; Baldi et al., 2001). A significant negative correlation between AP-2 expression level and tumor thickness was found and it was revealed that decreased AP-2 expression is independently associated with an elevated risk of subsequent metastatic behavior of stage I cutaneous malignant melanoma. Since AP-2 also regulates other genes that are involved in the progression of human melanoma such as c-KIT, E-cadherin, MMP2, and p21/WAF-1, it was proposed that loss of AP-2 is a crucial event in the development of malignant melanoma.

CREB

The camp-responsive element binding protein 1 (CREB1) is a basic/leucine zipper (bZip) transcription factor and binds the DNA sequence TGANNTCA, known as “cAMP responsive element” (CRE) (Barton et al., 1996). It has a molecular weight of 43 kDa. The CREB1 gene consists of 9 exons and is located on human chromosome 2q32.3-q34. CREB1 belongs to the ATF/CREB family combining several subgroups: CREB/CREM, CRE-BP1 (=ATF2), ATF3, ATF4, ATF6, and B-ATF (reviewed by Hai and Hartman, 2001).

Target genes of CREB1 include for example somatostatin, proenkephalin, fos and cyclin D1 (Boulon et al., 2002). The basal activity of CREB maps to a carboxy-terminal constitutive activation domain (CAD), whereas phosphorylation and inducibility map to a central, kinase-inducible domain (KID) (Quinn, 2002). Phosphorylation of the protein on serine residue 119 (Barton et al., 1996) and on serine residue 133 are critical for positive regulation of the transcriptional activity of CREB. In the unphosphorylated state, CREB binds DNA but cannot activate transcription. Phosphorylation of CREB on Ser133 facilitates its interaction with the CREB-binding protein (CBP) which in turn is able to interact with and activate the basal transcription complex (Fentzke et al., 1998).

Previously, Xie and co-workers (1997) reported that the expression of a dominant-negative CREB (KCREB), containing a mutated DNA-binding site, decreases the tumorigenicity and the metastatic potential of human MeWo melanoma cells in nude mice. Cells with diminished CREB expression displayed a significant decrease in MMP2 mRNA expression resulting in a reduced invasiveness of the cells. Also the cell-surface adhesion molecule MCAM/MUC18 was downregulated in KCREB-transfected melanoma cells. In additional experiments the role of CREB in inhibition of apoptosis was investigated using the agent thapsigargin (Tg). Tg induces apoptosis by increasing cytosolic Ca^{2+} via inhibition of endoplasmic reticulum-dependent Ca^{2+}ATPase. KCREB-transfected cells were more sensitive to Tg-induced apoptosis compared to mock-transfected cells. Furthermore, transcriptional activation of CREB by phosphorylation was observed in these cells (Jean et al., 1998b).

CtBP

C-terminal binding protein (CtBP) was originally purified as a protein specifically binding to the C-terminus of the adenoviral E1A protein (Boyd et al., 1993). CtBP1 is a phosphoprotein of approximately 48

kDa and is expressed throughout all developmental stages and in a wide variety of adult tissues (Furusawa et al., 1999). CtBP1 gene is localized on human chromosome 4p16 and consists of 9 exons.

This co-regulatory protein recognizes PXDLS motifs in DNA-binding proteins and functions as a transcriptional co-repressor in Drosophila, Xenopus and mammals, whereas CtBP proteins themselves cannot bind to DNA. A comprehensive summary of CtBP-interacting proteins is given in a review by Turner and Crossley (2001). The precise mechanisms as to how CtBP influences transcription are still under investigation. CtBP proteins dimerize and interact with histone deacetylases; hence they may operate by linking deacetylases to DNA-bound factors. It is also assumed that CtBP proteins exhibit intrinsic enzymatic activity because of a striking homology to dehydrogenase enzymes (Kumar et al., 2002) (Fig. 1). Recently, it has been shown that CtBP-binding to PXDLS motifs can be regulated by acetylation of residues neighbouring the motif (reviewed by Turner and Crossley, 2001).

In all assays, CtBP was shown to function as a transcriptional repressor via protein-protein-interactions (Furusawa et al., 1999; see also Fig. 4B). Binding of CtBP to proteins of the LEF/TCF family via the motif PXDLS was first experimentally proven in Xenopus (Brannon et al., 1999). Further studies revealed that TCF proteins mediate repression when bound to CtBP, CBP (CREB binding protein) and members of the Groucho family of transcriptional repressors (reviewed by Roose and Clevers, 1999). In Drosophila, dCtBP can interact with the proteins Hairy (Poortinga, 1998), Knirps, Kruppel, Snail, and Giant (Nibu et al., 1998a,b; Nibu and Levine, 2001). All are known repressors of gene activity and play an important role in early Drosophila embryogenesis.

Recently, our laboratory has been able to show that CtBP expression is lost in malignant melanoma (Poser et al., 2002). Melanocytes revealed CtBP expression on mRNA and protein levels whereas in melanoma cell lines and in melanoma tissue samples CtBP levels were strongly reduced. As CtBP was proven to be a transcriptional repressor of LEF/TCF, these data suggest that loss of CtBP leads to expression of LEF/TCF-controlled genes. Furthermore, we could prove that loss of wildtype CtBP in melanoma cells promotes expression of MIA (melanoma inhibitory activity) via TCF-4. MIA is a secreted protein which is only expressed in melanoma cells but not in melanocytes. It is a key molecule involved in progression and metastasis of malignant melanomas (for review please refer to Bosserhoff and Buettner, 2002).

Recent data from colon carcinoma revealed other TCF-dependent proteins such as MMP7 (Brabletz et al., 1999), Myc (van de Wetering et al., 2002), APCDD1 (Takahashi at al., 2002), COX2 (Araki at al., 2003) and Cyclin D1 (Tetsu and McCormick, 1999). Some of these genes (e.g. myc, MMP7) are already known to be upregulated in melanoma and it can be speculated that this is due to the activity of TCF. Experimental studies are needed to further clarify this correlation.

ETS-1

The oncogene v-ets was originally discovered as a component of the E26 genome, an avian leucosis virus. Up to date, several ETS family members are known (ETS-1, ETS2, ERG, ELK1, ELK2) which are highly conserved between species and all of them carry a so-called "ETS-domain" (Fig. 2). They activate transcription by interacting with purine-rich sequences (GGAA/T). ETS-1 is 51 kDa in size and regulated by phosphorylation. The Ets-1 gene is located on chromosome 11q23.3 and consists of 8 exons (reviewed by Seth et al., 1992).

Ets genes are involved in defined chromosomal translocations leading to the formation of chimeric fusion proteins that are associated with certain leukemias and soft tissue cancers. Ets genes also play a role in T-cell development and angiogensis (Sato, 2001). ETS-1 is known to positively regulate several genes that are important in tumor progression. Genes such as MMP1, MMP3, MMP9, uPA and beta 3 integrin were revealed to have ETS-1 binding sites in their promoter regions and were clearly shown to be induced by ETS-1 (Sato et al., 2001).

Unpublished data of our group suggest strong upregulation of ETS-1 in malignant melanomas whereas normal human melanocytes show no ETS-1 expression. As shown by RT-PCR, western blot and gel shift assays, human melanoma cells reveal constitutive and strong ETS-1 expression and activity.

Furthermore, it was shown that a polymorphism in the MMP1 promoter generating an additional ETS-1 binding site contributes to increased transcription of

Fig. 1. Protein structure of dCtBP. The three black boxes indicate the acid dehydrogenase homology domains in dCtBP. The activation region contains a conserved putative catalytic histidine residue (His315) (modified from Phippen et al., 2000).

Fig. 2. Schematic diagram of the ETS-1 protein. The well conserved DNA-binding ETS domain is located in the N-terminal region. Its flanking regions (301 – 331 and 415 – 440) exhibit autoinhibitory sequences. The MAP kinase phosphorylation site (Thr38) is also indicated. The pointed domain (PNT) is suggested to assist protein-protein-interactions. The central region of the protein contains putative transactivation domains (modified from Slupsky et al., 1998).

MMP1. Several studies indicate that expression of this polymorphism by tumor cells may provide a mechanism for more aggressive matrix degradation, thereby facilitating cancer progression (Rutter et al., 1998).

HMGB1

The high mobility group proteins (HMG) are highly conserved non-histone chromosomal proteins and up to now their expression is known to be abundant. This group of proteins is subdivided into HMG-1/-2, HMG-14/-17 and HMG-I/Y (review by Bustin et al., 1990). Recently, the most prominent family HMG-1/-2, has been renamed HMGB, comprising three proteins: HMGB1 (previously HMG1 or Amphoterin), HMGB2 (previously HMG2), and HMGB3 (previously HMG4 or HMG2b) (Muller et al., 2001).

Recently, HMGB1 became an object of special preference for researchers. The HMGB1 gene consists of 6 exons and is located on human chromosome 13q12. All members of the HMGB family have a molecular weight of approximately 25-30 kDa and share two homologous basic domains of about 75 amino acids (Fig. 3). This basic domain mediates DNA-binding and is named the "HMG box". Homologous domains are found in an increasing number of proteins regulating various cellular functions. A large list of proteins containing an "HMG box" domain (e.g. hUBF, LEF-1, SRY) as well as a description of the clustering relationship of the HMG superfamily has been summarized by Baxevanis and Landsman (1995).

The ability of "HMG box" proteins to cause unusual DNA structures like bends and loops are closely related to their DNA-binding activities. An "HMG box" domain is characterized by an L-shaped arrangement of three alpha-helices which bind to the minor groove of DNA causing a distortion of the path of DNA through as much as 130° (Bianchi and Lilley, 1995).

HMGB1 knockout mice are born alive, but die within 24 hours due to hypoglycemia. Cell lines lacking HMGB1 grew normally, but the activation of gene expression by glucocorticoid receptor (GR) is impaired (Calogero et al., 1999). This implies that HMGB1 is not essential for the overall organization of chromatin in the cell nucleus, but that it is critical for proper transcriptional control by specific transcription factors (e.g. Hox (Zappavigna et al., 1996), p53 (Jayaraman et al., 1998)). It is assumed that HMGB1 facilitates the formation of higher-order nucleoprotein complexes by bending DNA (review by Grosschedl et al., 1994).

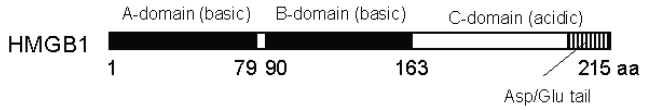


Fig. 3. Structure of the HMGB1 protein. Both domains A and B are highly basic and homologous to each other. Both display a so-called "HMG box" for DNA-binding via three alpha-helices. In contrast, the C-terminus contains a high proportion of acidic amino acids including a run of 30 aspartic and glutamic acid residues (related to Baxevanis and Landsman, 1995).

Recently, it was discovered that besides its intranuclear role, HMGB1 can also be secreted by certain cells. In this context, HMGB1 was implicated as a cytokine, because it is a late-acting mediator of endotoxin lethality that induces the release of pro-inflammatory cytokines from monocytes (Wang et al., 1999). Moreover, an important role of HMGB1 in tumor metastasis has been pointed out. A central cell surface receptor of HMGB1/Amphoterin in the developing brain is RAGE ("receptor for advanced glycation end products"). Engagement of RAGE by a ligand triggers activation of key cell signaling pathways, such as p21ras, mitogen-activated protein (MAP) kinases, NFκB and cdc42/rac, thereby reprogramming cellular properties. Blockade of RAGE-Amphoterin *in vitro* decreased growth and metastatic potential of both implanted tumors and tumors developing spontaneously in susceptible mice. Furthermore, suppressed activation of p44/p42, p38 and SAP/JNK MAP kinases were observed effecting important molecular mechanisms linked to tumor proliferation, invasion and expression of matrix metalloproteinases (Taguchi et al., 2000).

The expression pattern of HMGB1 was described to be ubiquitous, but a recent study by our laboratory documented HMGB1 to be strongly upregulated in melanoma cells in comparison to human primary melanocytes and revealed HMGB1 to play a role in melanoma development (Poser et al., 2003). In addition, we have been able to prove specific DNA-binding of HMGB1. These data are contrary to other findings that on the one hand say isolated HMG boxes retain their structural specificity of binding, but on the other that they only bind DNA unspecifically (Bianchi et al., 1992).

Our work reveals that HMGB1 binds to a specific region (Table 1) in the MIA (melanoma inhibitory activity) promoter and enhances promoter activity in melanoma cells. Repression of HMG expression in melanoma cells leads to a significant inhibition of MIA mRNA and protein expression and thus indirectly suppresses the ability of cell invasion. Furthermore, our data indicate for the first time that there is interaction of HMGB1 and p65 (NFκB) in a mammalian system (Poser et al., 2003). So far, this interaction has only been reported in Drosophila (Brickman et al., 1999; Decoville et al., 2000).

LEF /TCF / ß-catenin

The transcription factors of the LEF/TCF (lymphoid enhancer factor/T-cell-specific factor) family are the most downstream components of the Wnt signaling cascade. Today, four family members are known in mammals: LEF-1, TCF-1, TCF-3, and TCF-4. All share a homologous "HMG box" DNA-binding domain (Fig. 4A) and recognize the conserved consensus sequence

AGATCAAAGGG (van de Wetering et al., 1991; van Beest et al., 2000). For LEF-1, TCF-1, and TCF-4 several isoforms, due to alternative splicing, are described (van de Wetering et al., 1996; Duval et al., 2000; Hovanes et al., 2000). In contrast to the TCF family members only LEF-1 contains a context-dependent activation (CAD) domain (Carlsson et al., 1993; van de Wetering et al., 1996), which can activate transcription in the presence of the transcriptional co-activator ALY/BEF (Bruhn et al., 1997). Virbasius and co-workers (1999) identified and cloned a human nuclear protein that dramatically increases DNA-binding of transcription factors containing a basic leucine zipper (bZIP) DNA-binding domain. They showed that this protein, bZIP-enhancing factor (BEF), is identical to the murine ALY protein and functions as a molecular chaperone.

Although originally cloned as lymphoid transcription factors, members of the TCF family are now well accepted as key activators/repressors in many developmental processes. Unlike classical transcription factors, their binding to DNA alone is not sufficient to cause transcriptional activation. TCF family members become potent transactivators upon interaction with ß-catenin or its Drosophila counterpart Armadillo through activation of the Wnt signaling pathway.

Briefly, in the absence of Wnt signaling, ß-catenin levels in the cytoplasm are kept low due to the activity of a complex containing the serine/threonin kinase GSK3ß (glycogen synthase kinase 3 beta), the scaffold protein Axin and the tumor suppressor protein APC (adenomatous polyposis coli). ß-catenin bound to this complex becomes phosphorylated and is subsequently degraded by proteasomes in a ubiquitin-dependent manner. Following association of Wnt ligands to the transmembranous Frizzled receptor, this complex consisting of Axin, GSK3ß and APC is inactivated, resulting in accumulation of ß-catenin in the cytoplasm and its subsequent translocation into the nucleus. Here, ß-catenin functions as co-activator, binding to transcription factors of the LEF/TCF family, resulting in transactivation of target genes (reviewed by Polakis, 2000; Brantjes et al., 2002) (Fig. 4B).

In contrast, TCF proteins mediate repression when bound to members of the Groucho family of transcriptional repressors (in human named TLE-1 to 4 =

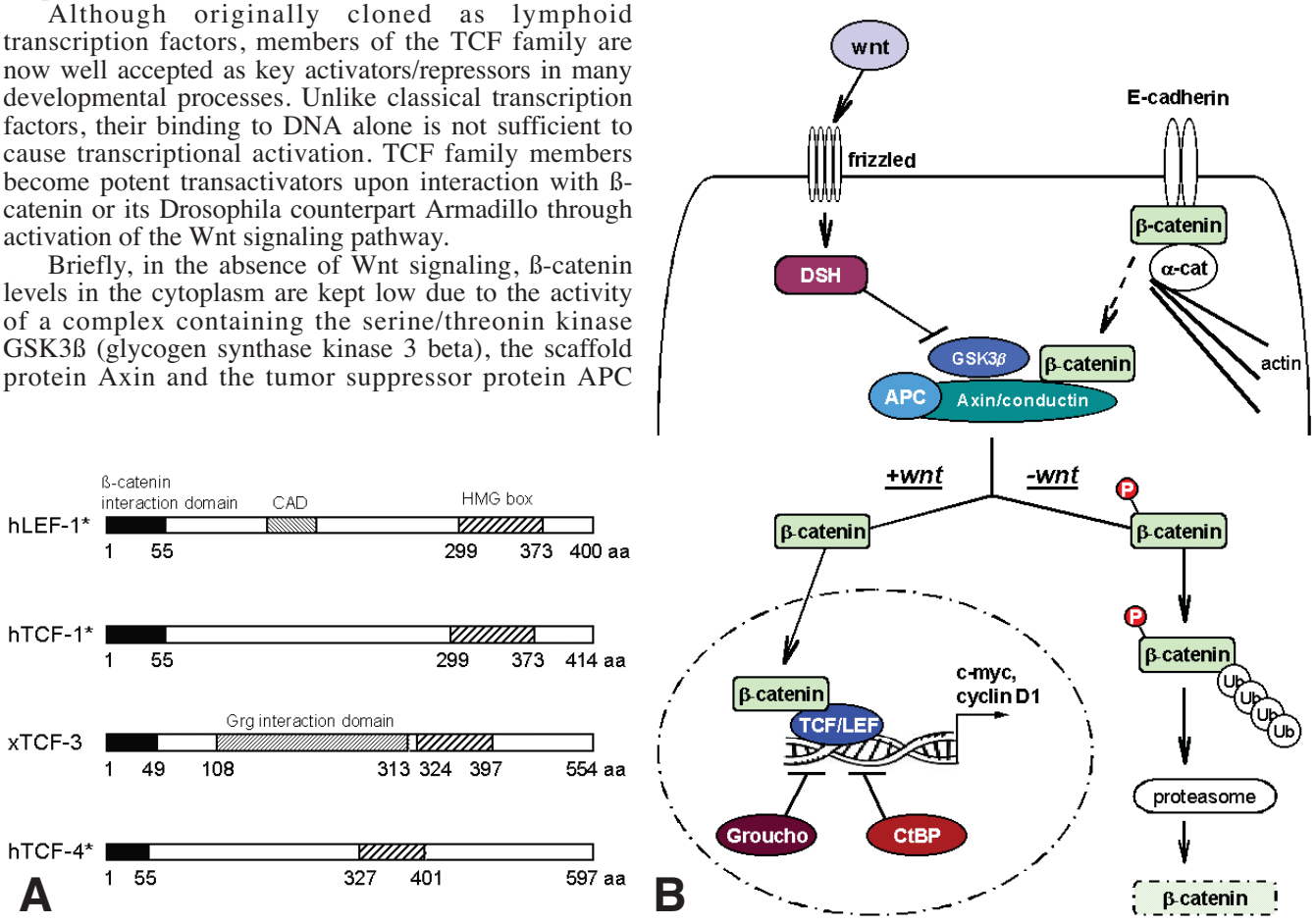


Fig. 4.A. Domain structure of transcription factors of the LEF/TCF family. All LEF/TCF proteins contain a conserved N-terminal ß-catenin-interacting domain and a centrally located DNA-binding "HMG box" domain. In LEF-1, both domains are connected by a region of lower degree of homology, a so-called "context-dependent activation domain" (CAD). In contrast, a Grg (Groucho) interaction domain has only been reported for TCF-3 so far. Asterisks indicate the existence of alternative splice products for LEF-1, TCF-1 and TCF-4 (modified from Roose and Clevers, 1999; Hovanes et al., 2000; Brantjes et al., 2002). **B.** Overview of the Wnt-signaling pathway. In the absence of Wnt (-wnt) ß-catenin complexes with APC, GSK3ß and axin/conductin, becomes phosphorylated by GSK3ß and subsequently multi-ubiquitinated (Ub) and degraded in proteasomes. Binding of Wnts to the frizzled receptor activates Dishevelled (DSH) which blocks the complex combining APC, GSK3ß and axin/conductin resulting in accumulation of cytoplasmic ß-catenin (+wnt). In direct consequence, ß-catenin translocates into the nucleus and associates with LEF/TCF transcription factors. The TCF/ß-catenin complexes bind to DNA and activate Wnt target genes. Binding of Groucho or CtBP (C-terminal binding protein) to TCF transcription factors represses activation of Wnt target genes. For details please refer to the "LEF/TCF section".

"transducin-like enhancer of split"), CBP (CREB binding protein) or CtBP. So far, only in Drosophila has the histone acetylase dCBP been shown to directly interact with TCF, resulting in acetylation of a conserved lysine in the ß-catenin binding domain of TCF leading to reduced affinity for binding of ß-catenin (Brantjes et al., 2002). Recently, TCF factors have been reported as tumor inducers, aberrantly activating their target genes as a result of elevated ß-catenin levels in many types of cancer (for a detailed review including phenotypes of LEF/TCF-deficient mice, please refer to Roose and Clevers, 1999). Especially in colon carcinoma these abnormal ß-catenin levels are predominantly caused by truncating mutations in the APC tumor suppressor gene (Powell et al., 1992) or stabilizing mutations in ß-catenin itself (Morin et al., 1997).

The involvement of the Wnt/ß-catenin pathway in progression of malignant melanoma is controversially discussed due to contradictory publications. In their publication, Rubinfeld and co-workers (1997) examined several melanoma cell lines and reported that the constitutive activation of a complex consisting of ß-catenin and LEF-1 is due to either mutant ß-catenin or mutant APC in these cell lines. However, other researchers could not confirm these findings (Rimm et al., 1999; Edmunds et al., 2002; Pollock and Hayward, 2002). For example, Rimm and co-workers (1999) showed that ß-catenin mutations are rare in primary malignant melanoma. In contrast, they frequently observed nuclear and/or cytoplasmic localization of ß-catenin, being a potential indicator of Wnt/ß-catenin pathway activation in melanoma. On the other hand, Weeraratna and co-workers (2002) reported that transfected melanoma cells constitutively overexpressing Wnt5a exhibited consistent changes including actin reorganization and increased cell adhesion. Interestingly, no increase in ß-catenin expression or nuclear translocation, but a dramatic increase in activated PKC was observed. In direct correlation with Wnt5a expression and PKC activation, there was an increase in melanoma cell invasion. Blocking this pathway using antibodies to Frizzled-5, the receptor for Wnt5a, PKC activity and cellular invasion was inhibited. Furthermore, Wnt5a expression in human melanoma biopsies directly correlates with increasing tumor grade.

Reifenberger and co-workers (2002) investigated 37 malignant melanomas (15 primary tumors and 22 metastases) for alterations of 4 genes encoding members of the Wnt pathway, i.e., CTNNB1 (ß-catenin gene), APC (adenomatous polyposis coli gene), BTRC (beta-transducin repeat-containing protein gene) and ICAT (inhibitor of beta-catenin and Tcf-4). Their results indicate only rare somatic mutations in ß-catenin, APC or ICAT, as well as low or absent expression of ICAT transcripts in 78% of the investigated tumors. Therefore, they assumed that this pathway may be altered in malignant melanomas by different mechanisms. Recently, Widlund and co-workers (2002) have been able to show that in melanoma cells ß-catenin is a potent mediator of growth via MITF (Microphthalmia-associated transcription factor), a downstream target of the Wnt pathway (please refer to "MITF"). As mentioned earlier when discussing CtBP, studies of our own laboratory have verified specific binding of TCF-4 to the MIA promoter and have documented its involvement in activating MIA expression in malignant melanoma due to the loss of CtBP expression (Poser et al., 2002).

Murakami and co-workers (2001) showed that LEF-1 mRNA expression is predominant in highly migrating cells from metastatic melanomas. These actively migrating melanoma cells showed nuclear and cytoplasmic accumulation of ß-catenin and active transcription of a reporter plasmid with LEF/TCF binding site.

MITF

Microphthalmia-associated transcription factor (MITF) is a basic helix-loop-helix leucine zipper protein functioning as a homodimer. It recognizes the AGTCATGTG DNA motif termed the "M-box" (Fig. 5A). The gene for human MITF is located on chromosome 3p14.1 – p12.3 and consists of 9 exons. Up to date four isoforms are known: MITF-A, -C, -H and -M. MITF-M is the shortest isoform consisting of 419 amino acids and is expressed specifically in neural-crest-derived melanocytes and melanoma cells (for review see Tachibana, 2000; Goding, 2000).

MITF is discussed as the master gene for melanocytic survival as well as the key transcription factor for regulation of melanogenic protein expression. MITF is known to regulate transcription of tyrosinase, TRP-1 and TRP-2, via binding to the "M-box" in the promoter region of each gene. Mice lacking MITF expression completely lose neural crest-derived melanocytes. MITF-M has not been shown to be oncogenic (Tachibana, 1997).

MITF appeared to be expressed without exception in malignant melanoma samples in a study by King et al. (1999), whereas Vachtenheimer and co-workers (1999) detected MITF expression only in some melanoma cell lines. This phenomenon seems to be due to repression in cultured cell lines. The role of mutations or abnormalities in the MITF gene in melanoma has not been evaluated up to date and needs to be elucidated.

A recent study revealed that MITF regulates Bcl-2 expression and thereby modulates cell survival (McGill et al., 2002). This is in agreement with a publication by Scholl and co-workers (2001), showing that downregulation of PAX3 in melanoma results in induction of apoptosis. Interestingly, Widlund and co-workers (2002) suggest that MITF is a target gene of the Wnt signaling pathway (please refer to "LEF/TCF/ß-catenin"). The Wnt pathway plays an integral role throughout development particularly in neural crest cells. Wnt has been shown to be necessary and sufficient to promote pigment cell fate during zebrafish

development. Induction of MITF expression by Wnt3a via ß-catenin and LEF/TCF has been demonstrated recently (Dorsky et al., 1998) (Fig. 5B).

NFκB

Nuclear factor of kappa light chain gene enhancer in B cells (NFκB) is activated by a wide variety of stimuli such as cytokines, radicals, ultraviolet irradiation, and bacterial or viral products. It plays a role in inflammatory events, apoptosis, cell growth and several other cellular processes (for reviews refer to Baeuerle and Henkel, 1994; Pahl, 1999). All target genes fall into four broad functional categories: immunoregulatory and inflammatory genes, anti-apoptotic genes, genes that positively regulate cell proliferation, and genes that encode negative regulators of NFκB. Genes of all four categories can contribute to tumorigenesis (review by Karin et al., 2002).

NFκB is a homo- or heterodimeric complex consisting of proteins of the Rel-family. Rel-proteins can be divided into two groups depending on their mode of synthesis and transactivation properties. One class combines p65 (also known as REL A), REL B and c-REL which are all directly synthesized in their mature forms. All share an amino-terminal REL homology domain (RHD) required for dimerization and DNA-binding and further transcription-modulating domains at their carboxy termini. The second class consists of p105 (also known as NFκB1) and p100 (also known as NFκB2) which are synthesized as large precursors with an N-terminal RHD and a C-terminal series of ankyrin repeats. This C-terminal domain is removed via ubiquitin-dependent proteolysis upon phosphorylation of p100 and p105, respectively. The mature proteins p52 and p50, respectively, only contain the RHD but lack transcription-modulating domains (Ghosh et al., 1998) (Fig. 6A). The most commonly detected dimers are p65/p50, p65/p65 and p50/p50. The DNA consensus sequence for binding of members of the NFκB family corresponds to GGGRNNYYCC (for review see Janssen-Heininger et al., 2000).

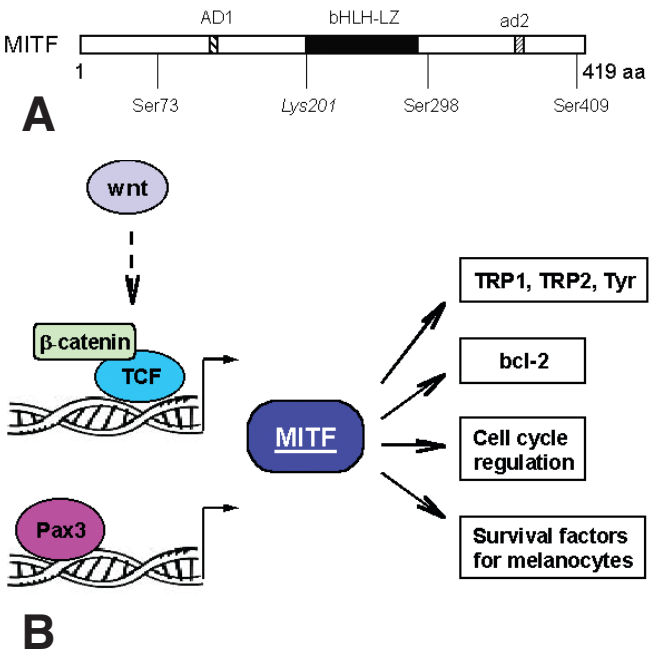


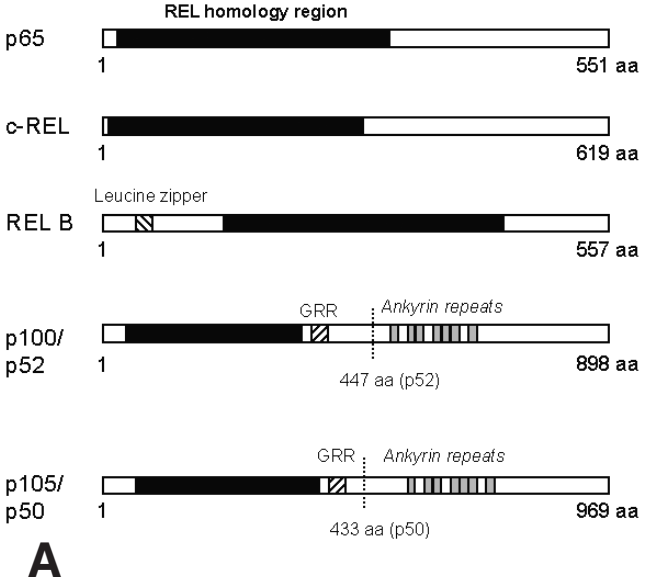
Fig. 5. A. Protein structure of the MITF protein. Dimerization and DNA-binding is mediated via the bHLH-LZ (basic helix-loop-helix/ leucine zipper) domain in the centre of the protein. The N-terminal transactivation domain AD1 interacts with the transcription adaptor proteins p300 and CBP. A second putative activation domain (ad2) is located in the C-terminal region. Three kinases influence MITF activation: the MAP kinase ERK2 targets Ser73; GSK3ß regulates MITF DNA-binding via phosphorylation on Ser298; and members of the p90 Rsk family modify Ser409. Phosphorylation of Ser78 and Ser409 regulates degradation of the protein via ubiquitination on Lys201 (modified from Goding 2000). **B.**Schematic diagram of MITF interactions. MITF is a target gene of the Wnt signaling pathway. Furthermore, PAX3 promotes MITF expression. Both mechanisms result in activation of MITF target genes which are involved in multiple processes including pigment production, cell survival (apoptosis) and proliferation.

Due to the presence of a strong transcriptional activation domain, p65 (REL A) is responsible for most of the NFκB transcriptional activity. The RHD of the proteins contains a nuclear localization signal (NLS) which is recognized by IκB proteins. They bind and inactivate NFκB by trapping the complexes in the cytoplasm. Normally, microbial and viral infections or exposure of proinflammatory cytokines activate the IkB kinase (IKK) complex (Fig. 6B). The IKK complex controls two distinct NFkB activation complexes. In one pathway, IKK beta phosphorylates two distinct serine residues (Ser32 and Ser36) of IkB alpha bound to NFκB heterodimers (p65/p65 and/or p65/p50) located in the cytoplasma. Subsequently IkB alpha is degraded via the ubiquitin-dependent pathway allowing nuclear translocation of NFκB dimers and transcriptional activation of target genes. In response to other stimuli such as lymphotoxin B (LTß) or BAFF, IKK alpha is activated to induce the phosphorylation of p100 bound to REL B at two serine residues at its C-terminus. Subsequently, ubiquitin-dependent degradation of the C-terminus of p100 is initiated and the mature p52 protein is released. Together with its heterodimeric partner REL B it translocates into the nucleus to activate gene transcription (for further details please refer to the review by Karin et al., 2002).

In malignant melanoma the members of the transcription factor family NFκB are constitutively active. Recently, the laboratory of Ann Richmond correlated the high level of activated NFκB to an increased IKK activity in melanoma cell lines. Consequently, constitutive activation of IKK leads to enhanced phosphorylation of IkBalpha and p65 followed by enhanced nuclear translocalization of p65/p50 in comparison to normal human epidermal melanocytes. Furthermore, the chemokines CXC ligand 1 (CXCL1) and CXCL8, but not CXCL5, are highly expressed in

most of the melanoma cell lines, suggesting that the constitutive production of chemokines is correlated to endogenous NFκB activity (Yang and Richmond, 2001). In addition to these findings, they also demonstrated that NFκB-inducing kinase (NIK) is strongly expressed in melanoma cells compared to primary melanocytes and increases phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2). The hypothesis that ERK acts upstream of NFκB and regulates the NFκB DNA-binding activity is supported by decreased NFκB promoter activity after overexpression of dominant negative ERK expression constructs. Taken together, these data indicate constitutive NIK-induced NFkB activation in malignant melanoma and involvement of MAP kinase signaling cascades in this process (Dhawan and Richmond, 2002).

Another study by our laboratory (Gutgemann et al., 2001) compared the gene expression of highly invasive and weakly invasive cell clones derived from the melanoma cell line Mel Im (Jacob et al., 1995, 1998). Both cell clones were selected using the Boyden Chamber model and showed a fivefold difference in their invasive potential *in vitro* and differences in tumorigenicity in an *in vivo* model, whereas the rate of cell proliferation and apoptosis remained unchanged after selection. Both array and real-time PCR showed upregulation of jagged2 (JAG2) mRNA expression in the highly invasive melanoma cell clones (Gutgemann et al., 2001). JAG2, together with Jagged1, Delta1 and Delta2, belongs to the DSL (Delta/Serrate/LAG-2) family of ligands for mammalian Notch receptors (for review see Weinmaster, 1997, 2000). Notch signaling has been implicated in a wide variety of processes including decisions of cell-fate, tissue patterning and morphogenic changes, all being involved in human diseases and cancer. In the human JAG2 promoter region, several potential binding sites for transcription factors, including NFκB, E47, E12, E2F, ETS-1, MyoD, and OCT-1 have been found (Deng et al., 2000). These data point to a correlation of upregulation of JAG2 and constitutive NFκB activity in malignant melanoma. In addition, Rel/NFκB was found to trigger the Notch signaling pathway by inducing expression of Jagged1 in splenic B-cells (Bash et al., 1999).



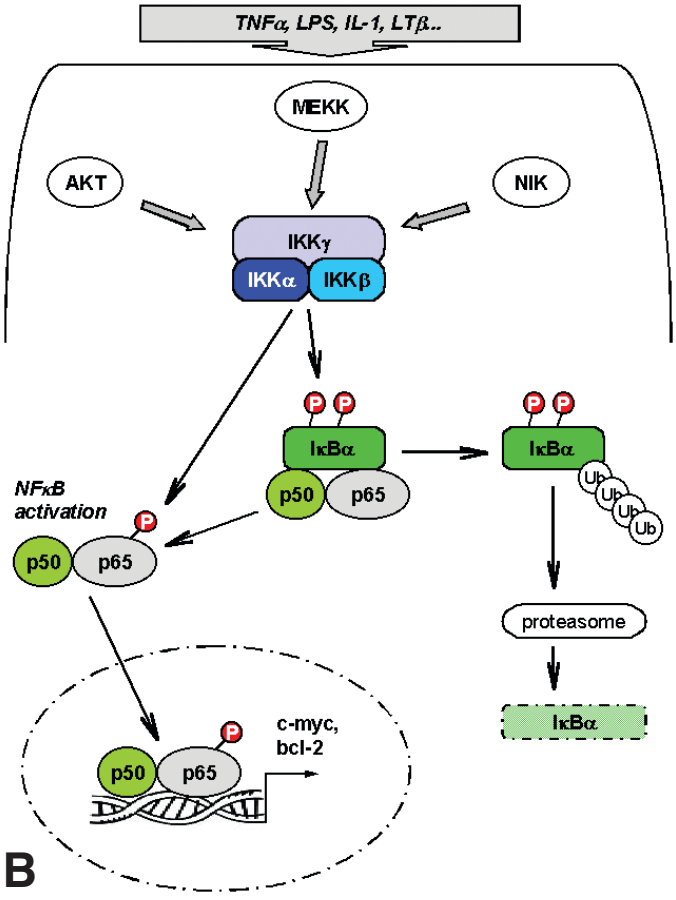


Fig. 6. A. Schematic diagram of NFκB proteins. All members of the transcription factor family of NFκB contain a large REL homology domain (RHD) for dimerization and DNA-binding (shaded in black). Additionally, there is a leucine zipper in the N-terminal region of REL B. The C-terminal halves of p100 and p105 are removed by ubiquitin-dependent degradation resulting in truncated p52 (447 aa) and p50 (433 aa). Both forms also contain a glycine-rich region (GRR) and ankyrin repeats in their C-terminal region (modified from Karin et al., 2002). **B.** Overview of the NFκB signaling pathway. In response to various stimuli, the complexing subunits of IkB kinases (IKK) are activated via the protein kinase AKT, MAP kinase kinases (MEKK) and/or NFκB-inducing kinase (NIK). This activation results in phosphorylation of IkB bound to NFκB subunits (p65/p50) and subsequent ubiquitin (Ub)-dependent degradation of IkB by proteasomes. This phosphorylation event triggers phosphorylation and therefore activation of released NFkB proteins (p65/p50) which translocate into the nucleus and transcriptionally activate NFκB target genes via binding to the NFkB concensus sequence. For details please refer to the "NFκB section".

PAX3

PAX (paired box) proteins are developmentally expressed transcription factors that play an important role in the establishment of cell lineages. Up to date nine human members of this family have been identified. The importance of PAX proteins was first seen in several loss-of-function mutations that led to a lack of specific structures or organs (Dahl et al., 1997). Paired box gene 3 (PAX3) is localized on human chromosome 2q35 and contains 4 exons. PAX3 was shown to strongly activate MITF in synergy with SOX10. Mutations in PAX3 are responsible for the Waardenburg syndrome which is characterized by pigmentation abnormalities and hearing impairment attributable to the absence of melanocytes. PAX3 expression was detected in all analyzed melanoma cell lines in a study by Vachtenheimer and Novotma (1999). This was irrespective of MITF expression and melanin production. This finding was confirmed in a study by Scholl and co-workers (2001). They further showed that PAX3 expression is important since downregulation resulted in apoptosis (Fig. 5B).

SKI

Ski and sno ("ski-related novel gene") were isolated by Nomura and co-workers (1989). Ski was originally discovered as an oncogene present in the avian Sloan-Ketterning Virus (SKV). The v-ski oncogene was found to cause morphological transformation in chicken embryos. C-Ski is ubiquitously expressed in developing mouse embryos, but elevated levels of Ski mRNA expression are found in differentiating muscle, lung and central nervous system. In humans, several isoforms of sno have been detected, designated as snoA, snoN, snoN2 and snoI (review by Liu et al., 2001). The c-ski gene was mapped to the human chromosome 1p36.3 and consists of 8 exons (Shinagawa et al., 2001) (Fig. 7A). Experiments determined a role of SKI in neural tube development and muscle differentiation. Overexpression of SKI rendered cells resistant to the growth inhibitory effects of TGF-beta. SKI cannot bind DNA by itself but was shown to function as a transcriptional repressor of SMAD3 in response to TGF-beta (Sun et al., 1999). SKI function is mediated by the I loop in the "SAND" domain of SMAD3 (Wu et al., 2002). Crystal structure analysis suggested that SKI disrupts the formation of a functional complex between the co-mediator SMADs (Co-SMADs) and receptor-mediated SMADs (R-SMADs).

It has been shown for the first time by Fumagalli and co-workers (1993) that SKI is overexpressed in malignant melanoma. The constant expression of c-Ski in the melanoma-derived cell lines at a level of expression much higher than that of normal melanocytes suggests that this proto-oncogene may play a role in melanocyte transformation. It has been known for a long time that melanoma cells are no longer sensitive to induced inhibition of proliferation by TGF-beta; they even start producing the factor themselves. Recently, this strange finding was explained in a publication by Xu and co-workers (2000). This group determined that SKI acts in opposition to TGF-beta-induced transcriptional

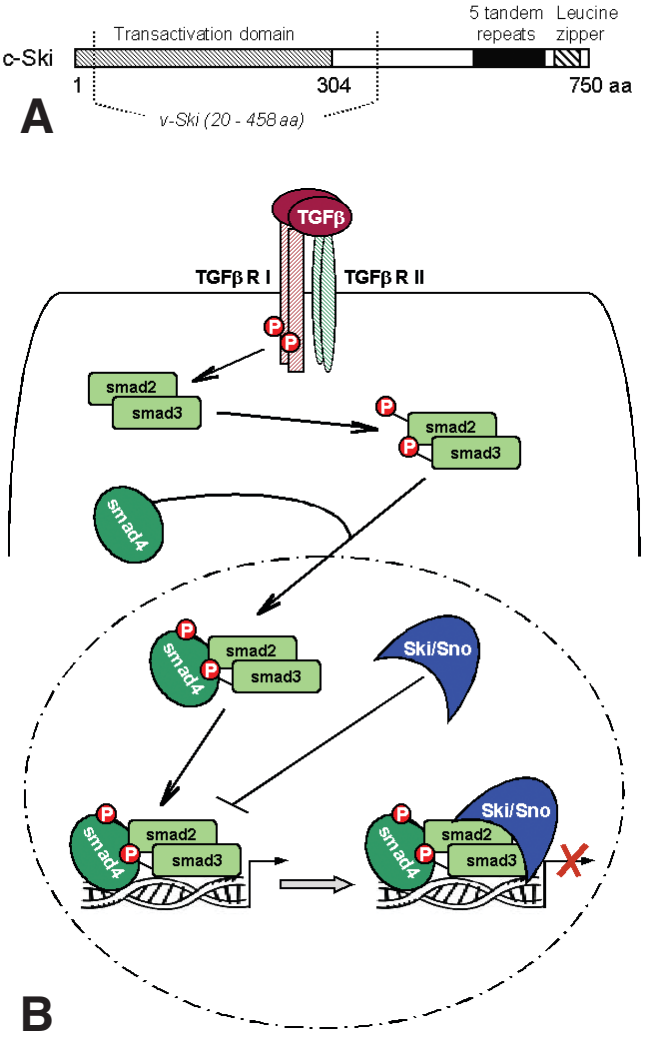


Fig. 7. A. Schematic structure of Ski proteins. Transcriptional regression of Ski proteins is mediated by the N-terminal "transformation domain" (Nicol et al., 1999). In v-Ski, a truncated form of Ski, the high affinity dimerization domain at the C-terminus of c-Ski is missing. This domain contains two distinct motifs: the first consists of 5 tandem repeats (25 amino acids each) forming an alpha-helix (Ser558 – Lys684); the second more C-terminal motif is a leucine zipper (Ser 686 – Asn750) that participates in dimer formation (modified from Zheng et al., 1997; Cohen et al., 1999). **B.** Interaction of Ski with the TGF-beta signaling pathway. Binding of TGF-beta to its receptors (TGF-beta RI and RII) results in phosphorylation of Smad2/3 complexes and the subsequent association with Smad4. Smad2/3/4 complexes translocate into the nucleus and activate TGF-beta-responsive genes. Normally, endogenous Ski/Sno is degraded proteasome-dependent in response to sTGF-beta stimulation, but in cells overexpressing Ski/Sno, excess Ski/Sno binds to the smad2/3/4 complex bound to the DNA and represses its activation of TGF-beta target genes. Therefore, Ski/Sno acts as an antagonist of TGF-beta-induced inhibition of cell proliferation.

activation by functioning as a Smad-dependent co-repressor. The biological relevance of this transcriptional repression was revealed by showing that overexpression of SKI abolished TGF-beta-mediated growth inhibition. Moreover, it was shown by immunohistochemical staining that SKI protein levels are increased in human melanoma tissues (Reed et al., 2001). In addition, SKI subcellular localization changes from nuclear, in preinvasive melanomas (melanomas in situ), to nuclear and cytoplasmic in primary invasive and metastatic melanomas. Furthermore, SKI/SMAD association in the cytoplasm seems to prevent SMAD3 nuclear translocation in response to TGF-beta (Fig. 7B).

Snail

Snail, a zinc finger transcription factor, was first identified in Drosophila where it was shown to be involved in the formation of the mesoderm. In this process, Snail acts as a transcriptional repressor downregulating the expression of ectodermal genes.

In the last years, Snail family members including Slug, Scratch, Smug, Escargot, Worniu and Ces1 have been found in humans and other vertebrates and non-vertebrate-like chordates, insects, nematodes, annelids and molluscs (for a detailed compendium please refer to the review by Nieto, 2002). All members of the Snail family are transcription factors expressing 4 to 6 zinc fingers in their conserved C-terminus. They promote specific DNA-binding to the "E-box" motif CANNTG which is also a recognition site for transcription factors of the basic helix-loop-helix (bHLH) family (Batlle et al., 2000) (Fig. 8). Snail expression in vertebrates was observed to be highest in embryonic kidney but expression was found in several other fetal tissues as well (Twigg and Wilkie, 1999). The snail gene is encoded by 3 exons situated on human chromosome 20q13.1.

Previously, it has been reported that expression of Snail proteins is also involved in the epithelial to mesenchymal transitions (EMT) responsible for the acquisition of invasiveness during tumor progression. This aspect of their activity is associated with their ability to directly repress E-cadherin transcription via binding to the E-cadherin promoter (Cano et al., 2000).

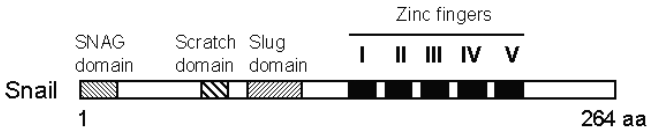


Fig. 8. Overall protein structure of members of the Snail superfamily. All family members of the Snail superfamily are characterized by a conserved C-terminal region consisting of 4 to 6 zinc fingers. They function as sequence-specific DNA-binding motifs recognizing "E-box" elements. In addition, the relative positions of the SNAG (Snail/Gfi) domain and the Scratch- and Slug-specific boxes are indicated. The SNAG domain facilitates transcriptional repression, but is missing in the Drosophila Snail without affecting its repressing abilities (modified from Nieto, 2002).

A study by our group revealed that upregulation of Snail is responsible for downregulation of the cell-cell-adhesion molecule E-cadherin in malignant melanoma (Poser et al., 2001). Normally, E-cadherin mediates strict growth control of melanocytes by keratinocytes in the epidermis and is presumed to act as a tumorsuppressor. Its loss is frequently observed in melanoma and it is known to contribute to tumor progression. In addition, E-cadherin has an important function in embryogenesis and tissue architecture by forming intercellular adherent junctions and establishing cell polarization. In a panel of different melanoma cell lines, E-cadherin expression was negatively regulated by upregulation of Snail. Transfection of human primary melanocytes with a Snail expression plasmid led to a 2.7-fold decrease in E-cadherin expression in these cells. Furthermore, stable transfection of an antisense Snail construct induced re-expression of E-cadherin in melanoma cells and indirectly reduced their invasive potential. All other known mechanisms of E-cadherin inactivation, e.g. mutations in the E-cadherin gene, promoter methylation or alterations in the expression of activating enhancer-binding protein 2 (AP-2) have been ruled out in malignant melanoma (Poser et al., 2001). Further studies are needed to reveal the mechanisms responsible for activated Snail expression in malignant melanoma and to discover other target genes.

Interestingly, the cytoskeletal protein Vimentin has been shown to be activated by Snail (Yokoyama et al., 2003) and is also frequently upregulated in melanoma cells compared with benign melanocytes. This correlates with the findings that TGF-beta-resistant fetal rat hepatocytes undergo EMT and show elevated Snail and Vimentin expression but lack Cytokeratin 18 and E-cadherin (Valdes et al., 2002). In squamous cell carcinoma Snail expression was found to induce MMP2 expression, giving the conclusion that Snail contributes to increased invasion not only through the inhibition of cell-cell adhesion but also through upregulation of MMP2 expression in these cells (Yokoyama et al., 2003).

Recently, the existence of an active human Snail retrogene, inserted within an intron of a novel evolutionarily conserved gene and expressed in different human tissues and cell lines, has been described and named SNAIL-like. Functional analyses in cell culture revealed that this retrogene maintains the potential to induce EMT, conferring migratory and invasive properties to epithelial cells (Locascio et al., 2002).

STAT

STAT proteins have a dual function in "signal transduction and activation of transcription" (Fig. 9A). Seven members of this family have been identified in mammalian cells: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. STATs are activated by tyrosine phosphorylation, form homo- or heterodimers

and translocate into the nucleus, where they bind to defined DNA sequence motifs differing between the individual STAT proteins (Fig. 9B). The first STAT protein cloned was STAT1 which is activated after ligand binding to the dedicated receptor. Ligands are for example interferon-gamma, interferon-alpha or interleukin-6 (IL-6). In contrast, STAT2 and STAT3 are activated by interferon-alpha but not by interferon-gamma. STAT 1 maps to human chromosome 2q32.2 whereas STAT2 maps to 12q13 and STAT3 maps to 17q21 (a detailed overview is given in the review by Benekli et al., 2003). It is assumed that STAT proteins can act as positive and negative regulators of transcription.

Pansky and co-workers (2000) studied 9 human melanoma cell lines with regard to growth inhibition by interferon-alpha and defects in intracellular signal transduction through the Jak (janus kinases)-STAT pathway. In 3 cell lines, a complete loss of growth restraint by interferon-alpha was detected. Moreover, they reported that in all cell lines different components of the Jak-STAT pathway were defective. Bohm and co-workers (2001) could also show that resistance of malignant melanoma cells to IL-6 was due to defects in the STAT signaling pathways. Their data suggest that resistance of advanced melanoma cells to interleukin-6 is associated with reduced inhibition of cyclin-dependent kinase 2 (CDK2), which appears to be a consequence of a complex alteration in interleukin-6 signal transduction by STAT pathways.

In a study by Niu and co-workers (2002), it was demonstrated that STAT3 is constitutively activated in the majority of human melanoma cell lines and tumor specimens examined. This seems to be regulated by c-Src tyrosine kinase, as blocking of Src tyrosine kinase activity, but not EGF receptor or JAK family kinases, results in inhibition of STAT3 signaling in melanoma cell lines. Interestingly, melanoma cells undergo apoptosis when either Src kinase activity or STAT3 signaling is inhibited. Blockade of Src or STAT3 is also accompanied by downregulation of expression of the anti-apoptotic genes Bcl-x(L) and Mcl-1. These findings demonstrate that Src-activated STAT3 signaling is important for the growth and survival of melanoma tumor cells.

Conclusion

The importance of transcription factors in development and progression of malignant melanoma has been shown by several groups and is summarized in this review. We are now beginning to understand this complicated network and are starting to dissect the relevant pathways. As transcription factors are hierarchically higher and have the ability to regulate many downstream targets, the understanding of their regulating mechanisms can give important insight into melanoma development. Moreover, understanding of the role and regulation of these transcription factors in

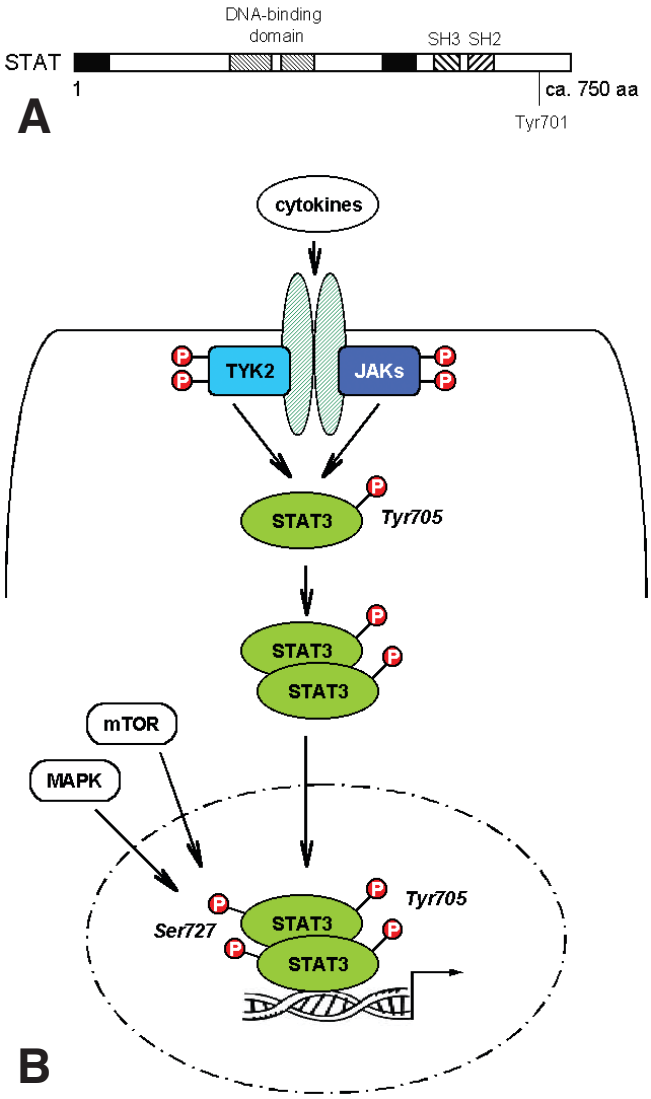


Fig. 9. A. Structure of STAT proteins. The most conserved region among the STAT proteins is an SH2 domain near the C-terminus which contains the phospho-tyrosine-binding arginine residue. A region similar to an SH3 domain is also indicated, recognising proline-rich motifs. Moreover, the critical tyrosine residue, Tyr701, is specified. Its phosphorylation is required for DNA-binding (Darnell et al., 1994). Boxes shaded in black indicate additional homologous regions among the members of the STAT family (modified from Ihle and Kerr, 1995; Benekli et al., 2003). **B.** Overview of the Jak/STAT signaling pathway. Ligand-induced receptor oligomerization activates protein-tyrosine kinase 2 (TYK2) and janus kinases (JAKs) which subsequently phosphorylate tyrosine residues (e.g. Tyr705) of the cytoplasmic part of the receptor. This results in recruitment of quiescent STAT monomers to the receptor and their phosphorylation by TYK2 and JAKs, respectively. Phosphorylation of STAT monomers allows them to dimerize and to translocate into the nucleus where they bind to specific DNA response elements and induce gene transcription. In addition, other pathways including mTOR (mammalian target of rapamycin) and MAP kinases, respectively, can phosphorylate (e.g. Ser727) and further activate STAT-dependent gene activation.

tumorigenesis will lead to future innovative therapeutic solutions.

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References

Araki Y., Okamura S., Hussain S.P., Nagashima M., He P., Shiseki M., Miura K. and Harris C.C. (2003). Regulation of cyclooxygenase-2 expression by the Wnt and ras pathways. Cancer Res. 63, 728-734.

Baeuerle P.A. and Henkel T. (1994). Function and activation of NF-kappa B in the immune system. Annu. Rev. Immunol.12, 141-179.

Baldi A., Santini D., Battista T., Dragonetti E., Ferranti G., Petitti T., Groeger A.M., Angelini A., Rossiello R., Baldi F., Natali P.G. and Paggi M.G. (2001). Expression of AP-2 transcription factor and of its downstream target genes c-kit, E-cadherin and p21 in human cutaneous melanoma. J. Cell Biochem. 83, 364-372.

Bar-Eli M. (2001). Gene regulation in melanoma progression by the AP-2 transcription factor. Pigment Cell Res. 14, 78-85.

Barton K., Muthusamy N., Chanyangam M., Fischer C., Clendenin C. and Leiden J.M. (1996). Defective thymocyte proliferation and IL-2 production in transgenic mice expressing a dominant-negative form of CREB. Nature 379, 81-85.

Bash J., Zong W.X., Banga S., Rivera A., Ballard D.W., Ron Y. and Gelinas C. (1999). Rel/NF-kappaB can trigger the Notch signaling pathway by inducing the expression of Jagged1, a ligand for Notch receptors. EMBO J. 18, 2803-2811.

Batlle E., Sancho E., Franci C., Dominguez D., Monfar M., Baulida J. and Garcia De Herreros A. (2000). The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat. Cell Biol. 2, 84-89.

Bauer R., Imhof A., Pscherer A., Kopp H., Moser M., Seegers S., Kerscher M., Tainsky M.A., Hofstaedter F. and Buettner R. (1994). The genomic structure of the human AP-2 transcription factor. Nucleid Acid Res. 22, 1413-1420.

Baxevanis A.D. and Landsman D. (1995). The HMG-1 box protein family: classification and functional relationships. Nucleic Acids Res. 23, 1604-1613.

Benekli M., Baer M.R., Baumann H. and Wetzler M. (2003). Signal transducer and activator of transcription proteins in leukemias. Blood 101, 2940-2954.

Bianchi M.E. and Lilley D.M. (1995). DNA-protein interactions. Applying a genetic cantilever. Nature 375, 532.

Bianchi M.E., Falciola L., Ferrari S. and Lilley D.M. (1992). The DNA-binding site of HMG1 protein is composed of two similar segments (HMG boxes), both of which have counterparts in other eukaryotic regulatory proteins. EMBO J. 11, 1055-1063.

Bohm M., Schulte U., Funk J.O., Raghunath M., Behrmann I., Kortylewski M., Heinrich P.C., Kues T., Luger T.A. and Schwarz T. (2001). Interleukin-6-resistant melanoma cells exhibit reduced activation of STAT3 and lack of inhibition of cyclin E-associated kinase activity. J. Invest. Dermatol. 117, 132-140.

Bosserhoff A.K. and Buettner R. (2002). Expression, function and clinical relevance of MIA (melanoma inhibitory activity). Histol. Histopathol. 17, 289-300.

Boulon S., Dantonel J.C., Binet V., Vie A., Blanchard J.M., Hipskind R.A. and Philips A. (2002). Oct-1 potentiates CREB-driven cyclin D1 promoter activation via a phospho-CREB- and CREB binding protein-independent mechanism. Mol. Cell Biol. 22, 7769-7779.

Boyd J.M., Subramanian T., Schaeper U., La Regina M., Bayley S. and Chinnadurai G. (1993). A region in the C-terminus of adenovirus 2 /5 E1a protein is required for association with a cellular phosphoprotein and important for the negative modulation of T24-ras mediated transformation, tumorigenesis and metastasis. EMBO J. 12, 469-478.

Brabletz T., Jung A., Dag S., Hlubek F. and Kirchner T. (1999). beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am. J. Pathol. 155, 1033-1038.

Brannon M., Brown J.D., Bates R., Kimmelman D. and Moon R. (1999). XCtBP is a XTcf-3 co-repressor with roles throughout Xenopus development. Development 26, 3159-3170.

Brantjes H., Barker N., van Es J. and Clevers H. (2002). TCF: Lady Justice casting the final verdict on the outcome of Wnt signalling. Biol. Chem. 383, 255-261.

Brickman J.M., Adam M. and Ptashne M. (1999). Interactions between an HMG-1 protein and members of the Rel family. Proc. Natl. Acad. Sci. USA 96, 10679-10683.

Bruhn L., Munnerlyn A. and Grosschedl R. (1997). ALY, a context-dependent coactivator of LEF-1 and AML-1, is required for TCRalpha enhancer function. Genes Dev. 11, 640-653.

Bustin M., Lehn D.A. and Landsman D. (1990). Structural features of the HMG chromosomal proteins and their genes. Biochim. Biophys. Acta 1049, 231-243.

Calogero S., Grassi F., Aguzzi A., Voigtlander T., Ferrier P., Ferrari S. and Bianchi M.E. (1999). The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. Nat. Genet. 22, 276-280.

Cano A., Perez-Moreno M.A., Rodrigo I., Locascio A., Blanco M.J., del Barrio M.G., Portillo F. and Nieto M.A. (2000). The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat. Cell Biol. 2, 76-83.

Carlsson P., Waterman M.L. and Jones K.A. (1993). The hLEF/TCF-1 alpha HMG protein contains a context-dependent transcriptional activation domain that induces the TCR alpha enhancer in T cells. Genes Dev. 7, 2418-2430.

Chiu R., Angel P. and Karin M. (1989). Jun-B differs in its biological properties from, and is a negative regulator of, c-Jun. Cell 59, 979-986.

Cohen S.B., Zheng G., Heyman H.C. and Stavnezer E. (1999). Heterodimers of the SnoN and Ski oncoproteins form preferentially over homodimers and are more potent transforming agents. Nucleic Acids Res. 27, 1006-1014.

Dahl E., Koseki H. and Balling R. (1997). Pax gene and organogenesis. BioEssays 19, 755-765.

Darnell J.E. Jr., Kerr I.M. and Stark G.R. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264, 1415-1421.

Decoville M., Giraud-Panis M.J., Mosrin-Huaman C., Leng M. and Locker D. (2000). HMG boxes of DSP1 protein interact with the rel homology domain of transcription factors. Nucleic Acids Res. 28, 454-462.

Deng T. and Karin M. (1993). JunB differs from c-Jun in its DNA-binding

and dimerization domains, and represses c-Jun by formation of inactive heterodimers. Genes Dev. 7, 479-490.

Deng Y., Madan A., Banta A.B., Friedman C., Trask B.J., Hood L. and Li L. (2000). Characterization, chromosomal localization, and the complete 30-kb DNA sequence of the human Jagged2 (JAG2) gene. Genomics. 63, 133-138.

Dhawan P. and Richmond A. (2002). A novel NF-kappa B-inducing kinase-MAPK signaling pathway up-regulates NF-kappa B activity in melanoma cells. J. Biol. Chem. 277, 7920-7928.

Dorsky R.I., Moon R.T. and Raible D.W. (2000). Control of neural crest cell fate by the Wnt signalling pathway. Nature 396, 370-373.

Duval A., Rolland S., Tubacher E., Bui H., Thomas G. and Hamelin R. (2000). The human T-cell transcription factor-4 gene: structure, extensive characterization of alternative splicing, and mutational analysis in colorectal cancer cell lines. Cancer Res. 60, 3872-3879.

Edmunds S.C., Kelsell D.P., Hungerford J.L. and Cree I.A. (2002). Mutational analysis of selected genes in the TGFbeta, Wnt, pRb, and p53 pathways in primary uveal melanoma. Invest. Ophthalmol. Vis. Sci. 43, 2845-2851.

Fentzke R.C., Korcarz C.E., Lang R.M. and Lin H., Leiden J.M. (1998). Dilated cardiomyopathy in transgenic mice expressing a dominant-negative CREB transcription factor in the heart. J. Clin. Invest. 101, 2415-2426.

Fumagalli S., Doneda L., Nomura N. and Larizza L. (1993). Expression of the c-ski proto-oncogene in human melanoma cell lines. Melanoma Res. 3, 23-27.

Furusawa T., Moribe H., Kondoh H. and Higashi Y. (1999). Identification of CtBP1 and CtBP2 as corepressors of zinc finfer-homeodomain factor delta-EF1. Mol. Cell Biol. 19, 8581-8590.

Ghosh S., May M.J. and Kopp E.B. (1998). NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu. Rev. Immunol. 16, 225-260.

Goding C.R. (2000). MITF from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage. Genes Dev. 14, 1712-1728.

Grigoriadis A.E., Wang Z.Q. and Wagner E.F. (1995). Fos and bone cell development: lessons from a nuclear oncogene. Trends Genet. 11, 436-441.

Grosschedl R., Giese K. and Pagel J. (1994). HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. Trends Genet. 10, 94-100.

Gutgemann A., Golob M., Muller S., Buettner R. and Bosserhoff A.K. (2001). Isolation of invasion-associated cDNAs in melanoma. Arch. Dermatol. Res. 293, 283-290.

Hai T. and Hartman M.G. (2001). The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. Gene 273, 1-11.

Hilger-Eversheim K., Moser M., Schorle H. and Buettner R. (2000). Regulatory roles of AP-2 transcription factors in vertebrate development, apoptosis and cell-cycle control. Gene 260, 1-12.

Hovanes K., Li T.W. and Waterman M.L. (2000). The human LEF-1 gene contains a promoter preferentially active in lymphocytes and encodes multiple isoforms derived from alternative splicing. Nucleic Acids Res. 28, 1994-2003.

Huang E.Y., Madireddi M.T., Gopalkrishnan R.V., Leszczyniecka M., Su Z., Lebedeva I.V., Kang D., Jiang H., Lin J.J., Alexandre D., Chen Y., Vozhilla N., Mei M.X., Christiansen K.A., Sivo F., Goldstein N.I., Mhashilkar A.B., Chada S., Huberman E., Pestka S. and Fisher P.B. (2001). Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (mda-7) gene with cancer specific growth suppressing and apoptosis inducing properties. Oncogene 20, 7051-7063.

Huang S., Jean D., Luca M., Tainsky M.A. and Bar-Eli M. (1998). Loss of AP-2 results in downregulation of c-KIT and enhancement of melanoma tumorigenicity and metastasis. EMBO J. 17, 4358-4369.

Ihle J.N. and Kerr I.M. (1995). Jaks and Stats in signaling by the cytokine receptor superfamily. Trends Genet. 11, 69-74.

Jacob K., Bosserhoff A.K., Wach F., Knuchel R., Klein E.C., Hein R. and Buettner R. (1995). Characterization of selected strongly and weakly invasive sublines of a primary human melanoma cell line and isolation of subtractive cDNA clones. Int. J. Cancer 60, 668-675.

Jacob K., Wach F., Holzapfel U., Hein R., Lengyel E., Buettner R. and Bosserhoff A.K. (1998). In vitro modulation of human melanoma cell invasion and proliferation by all-trans-retinoic acid. Melanoma Res. 8, 211-219.

Janssen-Heininger Y.M., Poynter M.E. and Baeuerle P.A. (2000). Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. Free Radic. Biol. Med. 28, 1317-1327.

Jayaraman L., Moorthy N.C., Murthy K.G., Manley J.L., Bustin M. and Prives C. (1998). High mobility group protein-1 (HMG-1) is a unique activator of p53. Genes Dev. 12, 462-472.

Jean D., Gershenwald J.E., Huang S., Luca M., Hudson M.J., Tainsky M.A. and Bar-Eli M. (1998a). Loss of AP-2 results in up-regulation of MCAM/MUC18 and an increase in tumor growth and metastasis of human melanoma cells. J. Biol. Chem. 273, 16501-16508.

Jean D., Harbison M., McConkey D.J., Ronai Z. and Bar-Eli M. (1998b). CREB and its associated proteins act as survival factors for human melanoma cells. J. Biol. Chem. 273, 24884-24890.

Jiang H., Lin J.J., Su Z.Z., Goldstein N.I. and Fisher P.B. (1995). Subtraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression. Oncogene 11, 2477-2486.

Karin M., Cao Y., Greten F.R. and Li Z.W. (2002). NF-kappaB in cancer: from innocent bystander to major culprit. Nat. Rev. Cancer 2, 301-310.

Karjalainen J.M., Kellokoski J.K., Eskelinen M.J., Alhava E.M. and Kosma V.M. (1998). Downregulation of transcription factor AP-2 predicts poor survival in stage I cutaneous malignant melanoma. J. Clin. Oncol. 16, 3584-3591.

King R., Weilbaecher K.N., McGill G., Cooley E., Mihm M. and Fisher D.E. (1999). Microphthalmia transcription factor: A sensitive and specific melanocyte marker for melanoma diagnosis. Am. J. Pathol. 155, 731-738.

Kumar V., Carlson J.E., Ohgi K.A., Edwards T.A., Rose D.W., Escalante C.R., Rosenfeld M.G. and Aggarwal A.K. (2002). Transcription corepressor CtBP is an NAD(+)-regulated dehydrogenase. Mol. Cell. 10, 857-869.

Liu X., Sun Y., Weinberg R.A. and Lodish H.F. (2001). Ski/Sno and TGF-beta signaling. Cytokine Growth Factor Rev. 12, 1-8.

Locascio A., Vega S., de Frutos C.A., Manzanares M. and Nieto M.A. (2002). Biological potential of a functional human SNAIL retrogene. J. Biol. Chem. 277, 38803-38809.

Madireddi M.T., Dent P. and Fisher P.B. (2000). AP-1 and C/EBP transcription factors contribute to mda-7 gene promoter activity during human melanoma differentiation. J. Cell. Physiol. 185, 36-46.

McGill G.G., Horstmann M., Widlund H.R., Du J., Motyckova G., Nishimura E.K., Lin Y.L., Ramaswamy S., Avery W., Ding H.F., Jordan S.A., Jackson I.J., Korsmeyer S.J., Golub T.R. and Fisher

D.E. (2002). Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. Cell 109, 707-718.

Morin P.J., Sparks A.B., Korinek V., Barker N., Clevers H., Vogelstein B. and Kinzler K.W. (1997). Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275, 1787-1790.

Muller S., Scaffidi P., Degryse B., Bonaldi T., Ronfani L., Agresti A., Beltrame M. and Bianchi M.E. (2001). New EMBO members' review: the double life of HMGB1 chromatin protein: architectural factor and extracellular signal. EMBO J. 20, 4337-4340.

Murakami T., Toda S., Fujimoto M., Ohtsuki M., Byers H.R., Etoh T. and Nakagawa H. (2001). Constitutive activation of Wnt/beta-catenin signaling pathway in migration-active melanoma cells: role of LEF-1 in melanoma with increased metastatic potential. Biochem. Biophys. Res. Commun. 288, 8-15.

Nakabeppu Y., Ryder K. and Nathans D. (1988). DNA binding activities of three murine Jun proteins: stimulation by Fos. Cell 55, 907-915.

Nibu Y. and Levine M.S. (2001). CtBP-dependent activities of the short-range Giant repressor in the Drosophila embryo. Proc. Natl. Acad. Sci. USA 98, 6204-6208.

Nibu Y., Zhang H., Bajor E., Barolo S., Small S. and Levine M. (1998a). dCtBP mediates transcriptional repression by Knirps, Kruppel and Snail in the Drosophila embryo. EMBO J. 17, 7009-7020.

Nibu Y., Zhang H. and Levine M. (1998b). Interaction of short-range repressors with Drosophila CtBP in the embryo. Science 280, 101-104.

Nicol R., Zheng G., Sutrave P., Foster D.N. and Stavnezer E. (1999). Association of specific DNA binding and transcriptional repression with the transforming and myogenic activities of c-Ski. Cell Growth Differ. 10, 243-254.

Nieto M.A. (2002). The snail superfamily of zinc-finger transcription factors. Nat. Rev. Mol. Cell Biol. 3, 155-166.

Niu G., Bowman T., Huang M., Shivers S., Reintgen D., Daud A., Chang A., Kraker A., Jove R. and Yu H. (2002). Roles of activated Src and Stat3 signaling in melanoma tumor cell growth. Oncogene 21, 7001-7010.

Nomura N., Sasamoto S., Ishii S., Date T., Matsui M. and Ishizaki R. (1989). Isolation of human cDNA clones of ski and the ski-related gene, sno. Nucleic Acids Res. 17, 5489-5500.

Pahl H.L. (1999). Signal transduction from the endoplasmic reticulum to the cell nucleus. Physiol. Rev. 79, 683-701.

Pansky A., Hildebrand P., Fasler-Kan E., Baselgia L., Ketterer S., Beglinger C. and Heim M.H. (2000). Defective Jak-STAT signal transduction pathway in melanoma cells resistant to growth inhibition by interferon-alpha. Int. J. Cancer 85, 720-725.

Pfisterer P., Ehlermann J., Hegen M. and Schorle H. (2002). A subtractive gene expression screen suggests a role of transcription factor AP-2 alpha in control of proliferation and differentiation. J. Biol. Chem. 277, 6637-6644.

Phippen T.M., Sweigart A.L., Moniwa M., Krumm A., Davie J.R. and Parkhurst S.M. (2000). Drosophila C-terminal binding protein functions as a context-dependent transcriptional co-factor and interferes with both mad and groucho transcriptional repression. J. Biol. Chem. 275, 37628-37637.

Polakis P. (2000). Wnt signaling and cancer. Genes Dev. 14, 1837-1851.

Pollock P.M. and Hayward N. (2002). Mutations in exon 3 of the beta-catenin gene are rare in melanoma cell lines. Melanoma Res. 12, 183-186.

Poortinga G., Watanabe M. and Parkhurst S.M. (1998). Drosophila CtBP: a Hairy-interacting protein required for embryonic segmentation and hairy-mediated transcriptional repression. EMBO J. 17, 2067-2078.

Poser I., Dominguez D., de Herreros A.G., Varnai A., Buettner R. and Bosserhoff A.K. (2001). Loss of E-cadherin expression in melanoma cells involves up-regulation of the transcriptional repressor Snail. J. Biol. Chem. 276, 24661-24666.

Poser I., Golob M., Weidner M., Buettner R. and Bosserhoff A.K. (2002). Down-regulation of COOH-terminal binding protein expression in malignant melanomas leads to induction of MIA expression. Cancer Res. 62, 5962-5966.

Poser I., Golob M., Buettner R. and Bosserhoff A.K. (2003). Upregulation of HMG1 leads to melanoma inhibitory activity expression in malignant melanoma cells and contributes to their malignancy phenotype. Mol. Cell Biol. 23, 2991-2998.

Powell S.M., Zilz N., Beazer-Barclay Y., Bryan T.M., Hamilton S.R., Thibodeau S.N., Vogelstein B. and Kinzler K.W. (1992). APC mutations occur early during colorectal tumorigenesis. Nature 359, 235-237.

Quinn P.G. (2002). Mechanisms of basal and kinase-inducible transcription activation by CREB. Prog. Nucleic Acid Res. Mol. Biol. 72, 269-305.

Reed J.A., Bales E., Xu W., Okan N.A., Bandyopadhyay D. and Medrano E.E. (2001). Cytoplasmic localization of the oncogenic protein Ski in human cutaneous melanomas in vivo: functional implications for transforming growth factor beta signaling. Cancer Res. 61, 8074-8078.

Reifenberger J., Knobbe C.B., Wolter M., Blaschke B., Schulte K.W., Pietsch T., Ruzicka T. and Reifenberger G. (2002). Molecular genetic analysis of malignant melanomas for aberrations of the WNT signaling pathway genes CTNNB1, APC, ICAT and BTRC. Int. J. Cancer 100, 549-556.

Rimm D.L., Caca K., Hu G., Harrison F.B. and Fearon E.R. (1999). Frequent nuclear/cytoplasmic localization of beta-catenin without exon 3 mutations in malignant melanoma. Am. J. Pathol. 154, 325-329.

Roose J. and Clevers H. (1999). TCF transcription factors: molecular switches in carcinogenesis. Biochim. Biophys. Acta 1424, M23-37.

Rubinfeld B., Robbins P., El-Gamil M., Albert I., Porfiri E. and Polakis P. (1997). Stabilization of beta-catenin by genetic defects in melanoma cell lines. Science 275, 1790-1792.

Rutberg S.E., Saez E., Lo S., Jang S.I., Markova N., Spiegelman B.M. and Yuspa S.H. (1997). Opposing activities of c-Fos and Fra-2 on AP-1 regulated transcriptional activity in mouse keratinocytes induced to differentiate by calcium and phorbol esters. Oncogene 15, 1337-1346.

Rutter J.L., Mitchell T.I., Buttice G., Meyers J., Gusella J.F., Ozelius L.J. and Brinckerhoff C.E. (1998). A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res. 58, 5321-5325.

Sato Y. (2001). Role of ETS family transcription factors in vascular development and angiogenesis. Cell Struct. Funct. 26, 19-24.

Sato Y., Teruyama K., Nakano T., Oda N., Abe M., Tanaka K. and Iwasaka-Yagi C. (2001). Role of transcription factors in angiogenesis: Ets-1 promotes angiogenesis as well as endothelial apoptosis. Ann. NY Acad. Sci. 947, 117-123.

Scholl F.A., Kamarashev J., Murmann O.V., Geertsen R., Dummer R. and Schäfer B.W. (2001). PAX3 is expressed in human melanoma and coontributes to tumor cell survival. Cancer Res. 61, 823-826.

Schorle H., Meier P., Buchert M., Jaenisch R. and Mitchell P.J. (1996). Transcription factor AP-2 essential for cranial closure and craniofacial development. Nature 381, 235-238.

Seth A., Ascione R. and Fisher R.J. (1992). The ets gene family. Cell Growth Diff. 3, 327-334.

Shinagawa T., Nomura T., Colmenares C., Ohira M., Nakagawara A. and Ishii S. (2001). Increased susceptibility to tumorigenesis of ski-deficient heterozygous mice. Oncogene 20, 8100-8108.

Slupsky C.M., Gentile L.N., Donaldson L.W., Mackereth C.D., Seidel J.J., Graves B.J. and McIntosh L.P. (1998). Structure of the Ets-1 pointed domain and mitogen-activated protein kinase phosphorylation site. Proc. Natl. Acad. Sci. USA 95, 12129-12134.

Sun Y., Liu X., Eaton E.N., Lane W.S., Lodish H.F. and Weinberg R.A. (1999). Interaction of the Ski oncoprotein with Smad3 regulates TGF-beta signaling. Mol. Cell. 4, 499-509.

Tachibana M. (1997). Evidence to suggest that expression of MITF induces melanocyte differentiation and haploinsufficiency of MITF causes Waardenburg syndrome type 2A. Pigment Cell Res. 10, 25-33.

Tachibana M. (2000). MITF: A stream flowing for pigment cells. Pigment Cell Res. 13, 230-240.

Taguchi A., Blood D.C., del Toro G., Canet A., Lee D.C., Qu W., Tanji N., Lu Y., Lalla E., Fu C., Hofmann M.A., Kislinger T., Ingram M., Lu A., Tanaka H., Hori O., Ogawa S., Stern D.M. and Schmidt A.M. (2000). Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature 405, 354-360.

Takahashi M., Fujita M., Furukawa Y., Hamamoto R., Shimokawa T., Miwa N., Ogawa M. and Nakamura Y. (2002). Isolation of a novel human gene, APCDD1, as a direct target of the beta-Catenin/T-cell factor 4 complex with probable involvement in colorectal carcinogenesis. Cancer Res. 62, 5651-5656.

Tetsu O. and McCormick F. (1999). ß-Catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature 398, 422-426.

Turner J. and Crossley M. (2001). The CtBP family: enigmatic and enzymatic transcriptional co-repressors. Bioessays 23, 683-690.

Twigg S.R. and Wilkie A.O. (1999). Characterisation of the human snail (SNAI1) gene and exclusion as a major disease gene in craniosynostosis. Hum. Genet. Oct. 105, 320-326.

Urabe A., Nakayama J., Taniguchi S., Terao H. and Hori Y. (1992). Expression of the c-fos oncogene in chemically-induced mouse tumours and in human skin tumours. J. Pathol. 168, 281-286.

Vachtenheimer J. and Novotma H. (1999). Expression of genes for microphthalmia isoforms, PAX3 and MSGI, in human melanomas. Cell. Mol. Biol. 45, 1075-1082.

Valdes F., Alvarez A.M., Locascio A., Vega S., Herrera B., Fernandez M., Benito M., Nieto M.A. and Fabregat I. (2002). The epithelial mesenchymal transition confers resistance to the apoptotic effects of transforming growth factor Beta in fetal rat hepatocytes. Mol. Cancer Res. 1, 68-78.

van Beest M., Dooijes D., van De Wetering M., Kjaerulff S., Bonvin A., Nielsen O. and Clevers H. (2000). Sequence-specific high mobility group box factors recognize 10-12-base pair minor groove motifs. J. Biol. Chem. 275, 27266-27273.

van de Wetering M., Oosterwegel M., Dooijes D. and Clevers H. (1991). Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box. EMBO J. 10, 123-132.

van de Wetering M., Castrop J., Korinek V. and Clevers H. (1996). Extensive alternative splicing and dual promoter usage generate Tcf-1 protein isoforms with differential transcription control properties. Mol. Cell. Biol. 16, 745-752.

van de Wetering M., Sancho E., Verweij C., de Lau W., Oving I., Hurlstone A., van der Horn K., Batlle E., Coudreuse D., Haramis A.P., Tjon-Pon-Fong M., Moerer P., van den Born M., Soete G., Pals S., Eilers M., Medema R., and Clevers H. (2002). The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 111, 241-250.

Virbasius C.-M.A., Wagner S. and Green M.R. (1999). A human nuclear-localized chaperone that regulates dimerization, DNA binding, and transcriptional activity of bZIP proteins. Mol. Cell. 4, 219-228.

Wang H., Bloom O., Zhang M., Vishnubhakat J.M., Ombrellino M., Che J., Frazier A., Yang H., Ivanova S., Borovikova L., Manogue K.R., Faist E., Abraham E., Andersson J., Andersson U., Molina P.E., Abumrad N.N., Sama A. and Tracey K.J. (1999). HMG-1 as a late mediator of endotoxin lethality in mice. Science 285, 248-251.

Weeraratna A.T., Jiang Y., Hostetter G., Rosenblatt K., Duray P., Bittner M. and Trent J.M. (2002). Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. Cancer Cell 1, 279-288.

Weinmaster G. (1997). The ins and outs of notch signaling. Mol. Cell. Neurosci. 9, 91-102.

Weinmaster G. (2000). Notch signal transduction: a real rip and more. Curr. Opin. Genet. Dev. 10, 363-369.

Widlund H.R., Horstmann M.A., Price E.R., Cui J., Lessnick S.L., Wu M., He X. and Fisher D.E. (2002). Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. J. Cell Biol. 158, 1079-1087.

Williams T., Admon A., Luscher B. and Tjian R. (1988). Cloning and expression of AP-2, a cell type specific transcription factor that activates inducible enhancer elements. Genes Dev. 2, 1557-1569.

Wu J.W., Krawitz A.R., Chai J., Li W., Zhang F., Luo K. and Shi Y. (2002). Structural mechanism of Smad4 recognition by the nuclear oncoprotein Ski: insights on Ski-mediated repression of TGF-beta signaling. Cell 111, 357-367.

Xie S., Price J.E., Luca M., Jean D., Ronai Z. and Bar-Eli M. (1997). Dominant-negative CREB inhibits tumor growth and metastasis of human melanoma cells. Oncogene 15, 2069-2075.

Xu W., Angelis K., Danielpour D., Haddad M.M., Bischof O., Campisi J., Stavnezer E. and Medrano E.E. (2000). Ski acts as a co-repressor with Smad2 and Smad3 to regulate the response to type beta transforming growth factor. Proc. Natl. Acad. Sci. USA 97, 5924-5929.

Yang J. and Richmond A. (2001). Constitutive IkappaB kinase activity correlates with nuclear factor-kappaB activation in human melanoma cells. Cancer Res. 61, 4901-4909.

Yokoyama K., Kamata N., Fujimoto R., Tsutsumi S., Tomonari M., Taki M., Hosokawa H. and Nagayama M. (2003). Increased invasion and matrix metalloproteinase-2 expression by Snail-induced mesenchymal transition in squamous cell carcinomas. Int. J. Oncol. 22, 891-898.

Zappavigna V., Falciola L., Helmer-Citterich M., Mavilio F. and Bianchi M.E. (1996). HMG1 interacts with HOX proteins and enhances their DNA binding and transcriptional activation. EMBO J. 15, 4981-4991.

Zheng G., Blumenthal K.M., Ji Y., Shardy D.L., Cohen S.B. and Stavnezer E. (1997). High affinity dimerization by Ski involves parallel pairing of a novel bipartite alpha-helical domain. J. Biol. Chem. 272, 31855-31864.

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