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/β-catenin, MITF, NFkB, 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.

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>
<thead>
<tr>
<th>TRANSCRIPTION FACTOR</th>
<th>CHROMOSOME</th>
<th>SIZE (kDa)</th>
<th>FUNCTION</th>
<th>DNA-BINDING DOMAIN</th>
<th>TARGET GENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP1 (c-fos)</td>
<td>14q24.3-q31</td>
<td>55</td>
<td>oncogene</td>
<td>TGA C/G TCA (&quot;TRE&quot;)</td>
<td>p21, mda-7, Fas, MMPC-7</td>
</tr>
<tr>
<td>(c-jun)</td>
<td>1p32-p31</td>
<td>43</td>
<td>oncogene</td>
<td>TGA C/G TCA (&quot;TRE&quot;)</td>
<td></td>
</tr>
<tr>
<td>AP2alpha</td>
<td>6p24-p22.3</td>
<td>52</td>
<td>activator</td>
<td>GCCNNNGGC</td>
<td></td>
</tr>
<tr>
<td>CREB</td>
<td>2q32.3-q34</td>
<td>43</td>
<td>activator</td>
<td>TGANNTCA (&quot;CRE&quot;)</td>
<td>Somatostatin, Proenkephalin, Fos, MMP2, MCAM/MUC18</td>
</tr>
<tr>
<td>ETS-1</td>
<td>11q23.3</td>
<td>51</td>
<td>activator/repressor</td>
<td>GGA A/T</td>
<td>MIA, MMP1, MMP9, uPA, β3 Integrin</td>
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<tr>
<td>HMGB1</td>
<td>13q12</td>
<td>32</td>
<td>activator/repressor</td>
<td>GGCATT (&quot;HCR&quot;)</td>
<td></td>
</tr>
<tr>
<td>LEF-1</td>
<td>4q22</td>
<td>55-57</td>
<td>activator/repressor</td>
<td>AGATCAAGGG</td>
<td>MMP7, Myc, APCDD1, SOX2, Cyclin D1, TCF-1, MITF</td>
</tr>
<tr>
<td>TCF-4</td>
<td>10q25.3</td>
<td>60</td>
<td>activator/repressor</td>
<td>AGTCAAGGG</td>
<td></td>
</tr>
<tr>
<td>MITF</td>
<td>3p14.3-p12.3</td>
<td>526 aa</td>
<td>activator</td>
<td>AGTCATGTG (&quot;M-box&quot;)</td>
<td>Trp1, Trp2, Tyr, Bcl-2</td>
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<tr>
<td>NFκB (p50)</td>
<td>4q23-q24 50</td>
<td>50</td>
<td>activator</td>
<td>GGRNNYYCC</td>
<td>c-myc, bcl-2</td>
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<tr>
<td>(p65)</td>
<td>11q12-q13 65</td>
<td>65</td>
<td>activator</td>
<td>GGRNNYYCC</td>
<td>c-myc, bcl-2</td>
</tr>
<tr>
<td>PAX3</td>
<td>2q35</td>
<td>215 aa</td>
<td>activator</td>
<td>TCGTCAGC G/C CNT/C/N/A</td>
<td>MITF</td>
</tr>
<tr>
<td>SKI</td>
<td>1p36.3</td>
<td>84</td>
<td>repressor</td>
<td>GTCTAGAC (&quot;SBE&quot;)</td>
<td>Smad3</td>
</tr>
<tr>
<td>Snail</td>
<td>20q13.1</td>
<td>264 aa</td>
<td>repressor</td>
<td>CANNTG (&quot;E-box&quot;)</td>
<td>E-cadherin, Vimentin</td>
</tr>
<tr>
<td>STAT1 (αβ)</td>
<td>2q32.2</td>
<td>91/84</td>
<td>activator/repressor</td>
<td>TTCNNNGAA (&quot;SIE-1&quot;)</td>
<td>p21/WAF1</td>
</tr>
<tr>
<td>STAT3 (αβ)</td>
<td>17q21</td>
<td>92/83</td>
<td>activator/repressor</td>
<td>TTCNNNGAA (&quot;SIE-1&quot;)</td>
<td>p21/WAF1</td>
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</tbody>
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<table>
<thead>
<tr>
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<th>SIZE (kDa)</th>
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<th>PROTEIN-BINDING DOMAIN</th>
<th>INTERACTING PROTEINS</th>
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<tbody>
<tr>
<td>CtBP</td>
<td>4p16</td>
<td>48</td>
<td>Co-repressor</td>
<td>none</td>
<td>PXDLS</td>
<td>TCF-3, TCF-4, delta-EF1, Hairy, Kruppel, Knirps, Snail, Giant, Cyclin D1, Evi-1</td>
</tr>
<tr>
<td>β-Catenin (CTNNB1)</td>
<td>3p22-p21.3</td>
<td>92</td>
<td>Co-activator</td>
<td>none</td>
<td>Domain of 59 aa on N-terminus of TCF</td>
<td>LEF/TCF; cadherin, catenin</td>
</tr>
<tr>
<td>SKI</td>
<td>1p36.3</td>
<td>84</td>
<td>repressor</td>
<td>none</td>
<td>&quot;SAND&quot; KDWK motif</td>
<td>Smad3</td>
</tr>
</tbody>
</table>

Transcription factors in melanoma development

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

Table 2. Overview of relevant transcription factors for melanoma development and progression acting via protein-protein interactions.
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 Ca2+ via inhibition of endoplasmic reticulum-dependent Ca2+/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
transcription factors in malignant melanoma

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 Levine, 1998). In Drosophila, dCtBP can interact with the proteins Hair (Porotnga, 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 et al., 2002), COX2 (Araki et 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 angiogenesis (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).](image-url)

![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).](image-url)
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 HMGB2b) (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 et al., 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 binding DNA (review by Grosschedl et al., 1994).

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, NFkB 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 effects 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 HMGB 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 (NFkB) 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 / β-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 β-catenin or its Drosophila counterpart Armadillo through activation of the Wnt signaling pathway.

Briefly, in the absence of Wnt signaling, β-catenin levels in the cytoplasm are kept low due to the activity of a complex containing the serine/threonin kinase GSK3β (glycogen synthase kinase 3 beta), the scaffold protein Axin and the tumor suppressor protein APC (adenomatous polyposis coli). β-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, GSK3β and APC is inactivated, resulting in accumulation of β-catenin in the cytoplasm and its subsequent translocation into the nucleus. Here, β-catenin functions as co-activator, binding to transcription factors of the LEF/TF family, resulting in transcriptional repression 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 =
Transcription factors in malignant melanoma

“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 β-catenin binding domain of TCF leading to reduced affinity for binding of β-catenin (Brantjes et al., 2002). Recently, TCF factors have been reported as tumor inducers, aberrantly activating their target genes as a result of elevated β-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 β-catenin levels are predominantly caused by truncating mutations in the APC tumor suppressor gene (Powell et al., 1992) or stabilizing mutations in β-catenin itself (Morin et al., 1997).

The involvement of the Wnt/β-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 β-catenin and LEF-1 is due to either mutant β-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 β-catenin mutations are rare in primary melanoma. In contrast, they frequently observed nuclear and/or cytoplasmic localization of β-catenin, being a potential indicator of Wnt/β-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 β-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 (β-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 β-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 β-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 β-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/β-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...
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 Baueuerle 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).

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

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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 NFκB 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 IκB 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 IκB bound to NFκB subunits (p65/p50) and subsequent ubiquitin (Ub)-dependent degradation of IκB by proteasomes. This phosphorylation event triggers phosphorylation and therefore activation of released NFκB proteins (p65/p50) which translocate into the nucleus and transcriptionally activate NFκB target genes via binding to the NFκB consensus 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 Novotna (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...

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**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).

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).

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 tumor suppressor. 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...
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References


Bianchi M.E., Falcioni 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.


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.


Transcription factors in malignant melanoma


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