Molecular biology of malignant melanoma and other cutaneous tumors

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SUMMARY

Skin cancer is the most common cancer worldwide. Its incidence is doubling every 15-20 years likely because of an aging population, changes in behaviour towards sun exposure, and increased UV light fluency at the earth surface due to ozone depletion. In this review, we summarize the most important genetic changes contributing to the development of malignant melanoma, basal cell carcinoma and squamous cell carcinoma, the main tumor entities arising in the skin. While our understanding of the oncogenes and tumor suppressor genes involved in the development and progression of skin tumors is still fragmentary, recent advances have shown alterations affecting conserved signalling pathways that control cellular proliferation and viability. These pathways include INK4a/Rb, ARF/p53, RAS/MAPKs, and sonic hedgehog/Gli.

INTRODUCTION

Skin is a complex tissue derived from both embryonic mesoderm and ectoderm that provides a barrier between the host and the environment. The epidermis, the outer layer of skin, is a multilayered squamous epithelium mostly formed by keratinocytes which is maintained through adult life by stem cells. Stem cells divide asymmetrically giving rise to one cell that remains as a stem cell (self-renewal) and another daughter cell that rapidly divides giving rise to a compartment of proliferating cells (transit amplifying cells). After a few cell divisions, all these cells undergo terminal differentiation and are shed constantly as dead keratinized squames (Fig. 1A). In addition to the keratinocyte, there are other three types of specialized cells in the epidermis: the melanin pigment- producing melanocyte,; the Langerhans cell that acts as an antigen-presenting cell in the lymph nodes; and the Merkel cell which is considered as an adapting mechanoreceptor (Fig. 1A). Besides preventing insensible loss of body fluids, the epidermis has the role to protect the body of environmental attacks, such as ultraviolet (UV) radiation, chemicals, viruses and other pathogens. As a result, epidermal cells have a high risk in acquiring gene mutations that could lead to the development of cancer. In particular, UV light exposure is the main responsible for the three major forms of human skin cancer: malignant melanoma, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). A fourth member of skin tumors that will be not included in this review is the rare but highly aggressive Merkel cell carcinoma[1].

UV light is divided into UVB (290-320 nm) and UVA (320-400 nm). UVC (< 290 nm) is completely absorbed by the atmosphere and is non-relevant for UV-induced skin carcinogenesis. Most of UVB light is also absorbed by ozone, but 5-10% of it reaches the earth surface. Notably, the exposure to the high penetrating UVB radiation leads to DNA lesions, such as the formation of cyclobutane pyrimidine dimer and pyrimidine pyridine photoproducts. The incorrect repair of these products produces DNA mutations (C to T and CC to TT transitions). UVA genotoxicity seems to be induced by indirect mechanisms mediated by reactive oxygen radicals[2]. In the skin, melanin is the pigment synthesized within specialized cytoplasmic organelles (melanosomes) in melanocytes. Melanin acts as a filter by absorbing solar radiation. Melanosomes are transferred to neighbouring keratinocytes within the epidermis, and the number, location and melanin content of the melanosomes within the keratinocytes determine skin and hair colour (see below and Fig. 1B).

INHERITED CUTANEOUS CANCER SYNDROMES

Inherited cancer syndromes have contributed significantly to the understanding of the genetic lesions involved in the genesis of both familial and sporadic forms of the disease. Two forms of inherited cancer syndromes are specific for skin tumors: familial melanoma and the nevoid basal cell carcinoma syndrome or Gorlin syndrome. In addition, a clear predisposition to develop all three forms of skin cancer is found in patients suffering xeroderma pigmentosum.

Familial melanoma

Familial melanomas represent about 8-12% of all melanoma cases. Linkage analysis studies of families with high melanoma incidence led to the identification of a locus at 9p21 in which the first melanoma susceptibility gene, *CDKN2A*, was identified. *CDKN2A* encodes two unrelated proteins: p16INK4a and p14ARF (p19 in mice) that are potent tumor suppressors involved in cell cycle regulation (Fig. 2A). p16INK4a specifically inhibits CDK4/CDK6-mediated phosphorylation of the retinoblastoma (Rb) protein arresting cell cycle progression through G1-S. p14ARF, on the other hand, enhances apoptosis and blocks oncogenic transformation by stabilizing p53 levels. Overall, germline *CDKN2A* mutations have been found in 20-40% of familiar melanomas. They comprise missense mutations found in exons 1α and exon 2, as well as in the 5' untranslated region and introns[3]. As *p16INK4a* shares exon 2 with *p14ARF* (see Fig. 2A) many *CDKN2A* mutations affect both proteins confounding the specific role of each gene in melanoma genesis. However, mutations affecting only *p14ARF* have been described in some melanoma families[4], thus pointing to *ARF* as a melanoma susceptibility gene that is independent of *INK4a*. In fact, genetically mouse models have provided convincing evidence that both *ARF* and *INK4a* are tumor suppressor genes in melanoma development[5]. In addition, genetic studies in melanoma families point to an additional unknown locus proximal to *CDKN2A* in the same chromosomal region as a risk gene for melanoma formation[4]. Other studies led to the finding of *CDK4* (located at 12q14) as a second melanoma susceptibility gene. Germline and sporadic mutations in *CDK4* abrogating binding of p16INK4a to CDK4 (Fig. 2A) have been found associated with melanoma pathogenesis. Thus, mutations of this gene have a similar impact to those in *p16INK4a*, and phenotypic characteristics of the families carrying *CDK4* germline mutations do not differ from *CDKN2A* affected families[6]. Consistent with the human data, mice expressing a mutant form of *cdk4* are predisposed to develop melanoma after carcinogen treatment[7].

It is well known that melanomas are most common in fair-skinned individuals with red or blond hair and freckling phenotype. Since certain melanocortin-1 receptor (*MC1R*) polymorphic variants are associated with these characteristics, this pigment regulating gene is seen as a risk modifier of melanoma. Moreover, *MC1R* variants increase melanoma penetrance in individuals harbouring *CDKN2A* germline mutations. The MC1R receptor is an important determinant for which type of melanin is produced by the melanocytes. Activation of MC1R by α-melanocyte-stimulating hormone (αMSH) leads to the expression of crucial pigmentary enzymes involved in melanin synthesis, such as tyrosinase, tyrosinase-related protein-1 (TRP-1) and dopachrome tautomerase (DCT) (see Fig. 1B). Most red hair phenotypes (and individuals who do not tan irrespective of the hair colour) have deficient MC1R function and are not able to synthesize the photoreactive eumelanin (black-brown pigment), but instead have more

of the photosensitizing and potentially mutagenic pheomelanin (reddish-yellow pigment)[4].

Nevoid basal cell carcinoma (Gorlin) syndrome

Our knowledge of the molecular biology of BCCs has come from the analysis of the nevoid basal cell carcinoma (NBCC) syndrome, also known as Gorlin syndrome. NBCC syndrome is an autosomal dominant disorder characterized by a wide range of developmental anomalies (i.e., neural and skeletal abnormalities) and predisposition to various cancers. These include medulloblastomas, meningiomas, rhabdomiosarcomas, ovarian and cardiac fibromas, fibrosarcomas, and significantly the development of multiple BCCs in the skin[8,9]. Obviously, the gene involved in this disease had to have a crucial role in both normal development and regulation of cell growth. This gene, mapped at 9q22-9q31, was identified as *PTCH1* due to its significant homology to *patched*, already known to play a key role in Drosophila embryogenesis as part of the hedgehog signalling pathway. Hedgehog signalling also plays a vital role in regulating patterning, proliferation, survival and growth in vertebrate embryos and adults[9]. Patched1 (ptch1), the protein encoded by *PTCH1* is a receptor of the secreted signalling glycoprotein sonic hedgehog (Shh, see Fig. 3). A *PTCH1* homologue, *PTCH2*, located at 1p32-1p34, was later identified but its function remains unclear. Germline loss-of function mutations of *PTCH1* leading to constitutive activation of the Shh signalling pathway have been found in patients with NBCC syndrome as well as in a substantial proportion of sporadic BCCs (Table 1). The majority of germline *PTCH1* mutations in NBCC patients are rearrangements resulting in truncated proteins. As NBCC patients normally inherit a mutated copy of the *PTCH1* gene, tumors are likely to arise after inactivation of the remaining allele.

Xeroderma pigmentosum

A predisposition to skin cancer is also associated with the rare hereditary syndrome xeroderma pigmentosum (XP). Individuals with XP carry a nucleotide excision DNA repair defect associated with an acute photosensitivity. The most significant characteristic of XP patients is a predisposition to develop multiple skin cancers, mostly SCCs but also BCCs and malignant melanomas. These tumors arise early in age on sun-exposed skin areas, and present higher levels of UV-specific mutations in key regulatory genes than the same tumors from individuals with normal nucleotide excision repair mechanisms[10,11]. A proportion of severely affected XP patients also exhibit neurological disorders and higher frequencies of brain tumors.

MALIGNANT MELANOMA

It is believed that melanoma, the most aggressive skin tumor, results from intense intermittent UV light exposure, along with severe sunburn during childhood[2,12]. In contrast to BCCs, malignant melanomas (and also SCCs, see below) seem to develop as a multi-step process. Melanomas are histologically classified into five distinct stages: common acquired and congenital nevi without dysplasia, dysplastic nevi, radial-growth phase (RGP) melanoma, vertical-growth phase (VGP) melanoma and metastatic melanoma (Fig. 4A). RGP melanoma grow laterally and remains largely confined to the epidermis, while VGP melanoma invades the upper layers of the epidermis and penetrates into the underlying dermis and subcutaneous tissue forming expansile

nodules of malignant cells. It is believed that the crucial step in the evolution to malignant melanoma is the transition from RGP to VGP melanoma[4].

Despite its important role in melanoma predisposition, mutations in *CDKN2A* are rarely found in sporadic primary melanomas. In contrast, *CDKN2A* mutations are found frequently in melanoma cell lines. This discrepancy could reflect a selective event imposed by cell culturing due to the critical role of p16INK4a in senescence, as cells that lose p16INK4a escape senescence and become immortalized[3]. Activating mutations in protooncogenes leading to constitutive activation of the mitogen-activated protein kinase (MAPK) signalling cascade have been found in melanocytic lesions. Strikingly, mutations in *BRAF* resulting in constitutive activation of this serine/threonine kinase have been found in about 60% of melanoma samples and cell lines. RAF proteins are the primary mediators of RAS signalling, which links extracellular mitogenic stimuli to transcription of growth related genes via the MAPK pathway (Fig. 2B). Most of these *BRAF* mutations occur at a single site leading to the substitution of valine by glutamic acid (V600E) in the kinase domain[3]. *BRAF* mutations are common in benign and dysplastic nevi pointing to a potential initiating role of BRAF in melanocyte transformation[4]. There have been also reports documenting mutations in *RAS* genes in melanocytic tumors. The most frequently mutated member of the family is *NRAS*, while mutations in *HRAS* and *KRAS* have only been found occasionally[3]. Activating point mutations in *NRAS* have been reported in as many as 56% of congenital nevi, 33% of primary melanomas and 26% of metastatic melanoma samples, but are rarely found in dysplastic nevi. This fact indicates the possible existence of two distinct evolutionary paths to melanoma progression, from benign and dysplastic nevi[4] (Fig. 4A). Interestingly, *NRAS* and *BRAF* activating mutations are mutually exclusive, indicating that these genes function in the same cellular growth regulatory pathway.

Other studies support aberrant hepatocyte growth factor/scatter factor (HGF/SF) signalling associated with melanoma progression. Increased expression of c-MET, the tyrosine kinase receptor of HGF/SF, has been observed in metastatic melanoma. In addition, gain of the 7q33-qter locus (where *c-MET* is located) has been correlated with late stages of melanoma development[4]. Besides to stimulate proliferation and motility of cultured melanocytes, HGF/SF disrupts adhesion between melanocytes and keratinocytes by downregulating the expression of the cell-cell adhesion proteins E-cadherin and desmoglein-1, which could favours deregulated cell proliferation and invasiveness.

PTEN encodes a lipid and protein phosphatase involved in negative regulation of the phosphatidylinositol 3-kinase (PI3K)-AKT signalling pathway. *PTEN* is among the tumor suppressor genes most frequently mutated in cancer. Involvement of *PTEN* in melanoma was suspected because LOH of 10q (where *PTEN* is located) occurs in 30-50% of melanomas. Loss of *PTEN* leads to activation of the PI3K-AKT pathway that transmits potent cell proliferation and survival signals. The region deleted at 10q is, however, large and could include other tumor suppressor genes[3]. Several authors have reported that the overall mutation frequency of *PTEN* in primary melanomas and metastases is 5-15%[3,4]. The frequency of *PTEN* mutations increases up to 30-40% in melanoma cell lines, paralleling the difference observed for *CDKN2A* between tumors and cultured cells.

Interestingly, the proportion of primary melanomas containing mutations in the gene encoding p53 (*TP53*) is consistently low (<5%). Although UV light-related *TP53* mutations are frequently observed in BCCs and SCCs (see below), the above observation suggests that UV-induced mutational inactivation of p53 is not involved in melanoma formation.

Recent studies with high resolution of array comparative genomic hybridization (CGH) platforms have revealed that the evolution of melanoma is shaped by extensive chromosomal rearrangements, particularly recurrent chromosomal gains/amplifications and losses/deletions, and that distinct genomic signatures correlate with melanomas arising in different anatomical sites with different UV exposure patterns[13]. These studies, in conjunction with gene-specific mutational analyses will provide more accurate classification schemes and help to design rational therapeutic approaches.

BASAL CELL CARCINOMA

BCC is the most frequent skin tumor and the most common form of human cancer. As in melanoma, intense intermittent exposure to the sun during childhood and adolescence is associated with a higher risk for developing BCCs. Besides UV light, other risk factors, such as ionizing radiation, arsenic, psoralens and immunosuppression in organ transplant patients, have also been linked to the development of BCC[14]. BCCs rarely metastasize (< 0.1%) and are characterized by lack of chromosomal instability[15], making them unusual carcinomas. Nevertheless, BCCs can be highly invasive locally and can cause massive tissue damage. It is now generally accepted that BCCs are hair follicle-derived tumors and that they result from inappropriate activation of the Shh-Gli signalling pathway (Fig. 3) in hair follicle keratinocytes. Thus, overexpression of Gli1, Gli2 and Ptch1 (all of them targets of Shh signalling, see the legend of Fig. 3) has been consistently found in BCCs[10].

Mutations in *PTCH1* (either rearrangements resulting in a truncated protein or point mutations leading to premature protein termination) are relatively frequent in sporadic BCCs[10]. In contrast, mutations in *SHH* are extremely rare in these tumors (Table 1). The mutational spectrum in the *PTCH1* gene indicates a major role of UV light in the development of sporadic BCCs, as about 50% of these mutations are UVB-specific C to T or CC to TT. Loss of the wild type allele has been demonstrated in up to 68% of sporadic tumors, in accordance with a tumor suppressor mechanism for *PTCH1*[16]. Interestingly, mutations in the *SMO* gene have also been found in sporadic BCCs. The majority of *SMO* mutations are located in the extracellular domains of the Smo protein. These mutations may alter the latent state of Smo (by releasing Ptch1 inhibition) and result in constitutive activation of Shh signalling. Therefore, *SMO* can be viewed as a protooncogene and, in fact, Smo mutants cooperate with other oncogenic proteins in transformation of rat embryo fibroblasts. In BCCs of XP patients the frequency of mutations in the different partners involved in the Shh pathway are substantially increased, particularly that of *PTCH1* (Table 1). Genetically engineered mouse models for different partners of the Shh pathway strongly indicate that activation of Shh signalling is sufficient for BCC formation[9,10]. Thus, overexpression of Shh, Gli1 or Gli2 in the skin of transgenic mice leads to BCC-like tumors. In addition, human keratinocytes overexpressing Shh also display abnormal BCC-like structures when grafted onto immune deficient mice. Interestingly, *ptch1*$^{+/-}$ knock-out heterozygous mice do not develop BCCs but only small follicular tumors. Nevertheless, *ptch1*$^{+/-}$ mice do develop BCC-like lesions after being exposed to UV or ionizing radiation[17].

Mutations in the *TP53* tumor suppressor gene have been found in approximately 50% of sporadic BCCs. Although these mutations show the UVB signature its relevance for the development of BCC is not clear at present[8,15]. Several data suggest that p53 mutations in BCCs are secondary events that do not contribute significantly to tumorigenesis, while other authors postulate that p53 mutations are crucial but a late event in tumor progression[15].

SQUAMOUS CELL CARCINOMA

In contrast to BCC, cutaneous SCC is an aggressive tumor that may metastasize (commonly at lymph nodes) at frequencies reported between 1% and 12.5%. It is believed that SCCs arise from basal keratinocytes of the interfollicular epidermis in chronically sun-exposed skin, as these tumors are characterized by squamous differentiation. Whereas early-age recreational and intermittent intense sun exposure has been linked to BCC and malignant melanoma, high cumulative lifetime sun exposure has been associated with a higher risk for developing SCC[18,19]. The incidence of SCC is also high in patients receiving immunosuppressive therapy, a process that may be enhanced by concomitant human papilloma virus (HPV) infection. In individuals with intact immune system, however, there is no clear evidence for a role of HPV in the pathogenesis of this tumor. SCC develops as a classical cancer through well defined histological precancerous stages. Actinic keratoses (AK), a focal dysplasia of epidermal keratinocytes, and SCC *in situ* (CIS, also known as Bowen disease), a severe dysplasia, are preinvasive stages of invasive SCCs (Fig. 4B). Another skin lesion which is controversially discussed in this sequence of tumor evolution is the keratoacanthoma, a benign squamous neoplasm characterized by a rapid growth phase and then spontaneous regression after several months[20].

The *TP53* locus appears to play a significant role in the pathogenesis of SCC. p53 is involved in the induction of epidermal cell apoptosis following UV radiation-mediated DNA damage[21]. Therefore, the loss of p53 provides a survival advantage to UV-damaged keratinocytes. *TP53* is mutated in 60-90% of SCCs and AKs, and the mutations often display a typical UVB signature (C to T or CC to TT). *TP53* point mutations appear to arise early and are often found in cases with mild or moderate dysplasia. LOH affecting the *TP53* locus (which is located at 17p13.1) is more common at later stages, likely associated with the transition from AK to CIS[19]. In addition, a number of other chromosomes have been shown to carry LOH in SCCs, mostly restricted to 3p, 9p, 9q, 13q, 17p and 17q[20]. Despite the important role played by p53 in the development and progression of sporadic SCCs, it is interesting to note that individuals suffering the Li-Fraumeni syndrome due to a germline inherited mutation in *TP53* do not appear to be at increased risk for SCC[22]. In contrast, these patients are prone to develop a wide variety of internal neoplasms, including sarcomas, breast cancer, brain tumors, and lymphomas, in accordance with *TP53* being the most important tumor suppressor gene in human cancer. Mutations at lower frequencies than that of *TP53* have also been found in other cancer-related genes. Thus, UV-induced mutations in *HRAS* and *KRAS* have been characterized in both AKs and SCCs[22].

Progression from AK to SCC has been suggested to correlate with deletion of *CDKN2A*, and immunohistochemical analysis demonstrating the presence of p16INK4a in AK but its absence in SCC supports these LOH studies[19]. However, a recent study has shown that promoter methylation might be the predominant mechanism for inactivation of both p16INK4a and p14ARF in sporadic SCCs[23]. It should be emphasized, in this regard, that concomitant inactivation of these two non-overlapping pathways, ARF/p53 and INK4A/Rb, must be cooperative in promoting tumorigenesis.

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Legends to Figures

Figure 1. (A) Schematic representation of the epidermis showing the main cell types forming this tissue. The epidermis is spatially organized in different cell layers. Keratinocyte proliferation takes place on the basal layer. Most spinous cells have lost the ability to divide but they are metabolically active. Granular cells occupy an intermediate position between spinous cells and metabolically inactive cornified cells (squames) of the stratum corneum. (B) Biochemical pathways leading to the synthesis of melanins. TRP-1, tyrosinase-related protein-1; DCT, dopachrome tautomerase (also called TRP-2).

Figure 2. (A) *CDKN2A* locus. The *CDKN2A* gene encodes two proteins p16INK4a and p14ARF. Each has a unique first exon (1β or 1α) that then splices to a common second and third exon, but in alternate reading frames. p16INK4a binds and inhibits the activities of the cyclin-dependent kinases CDK4 and CDK6 ensuring that Rb remains in a hypophosphorylated state in complex with E2F transcription factor, leading to G1 arrest. p14ARF stabilizes and enhances p53 levels by inhibiting MDM2-mediated p53 ubiquitylation and degradation through the proteasome. p53 accumulation leads to either cell cycle arrest or cellular apoptosis. (B) The RAS/MAPK signalling pathway. After binding of growth factors to their respective receptor tyrosine kinase (RTK), activation of RTKs leads to binding of SOS (a cytosolic protein in close proximity to RAS on the plasma membrane) to the GDP-bound inactive form of RAS. Like other G proteins, RAS cycles between the GDP-bound inactive form and the GTP-bound active form. The binding of SOS to RAS induces a conformational change that leads to the dissociation of GDP and binding of GTP. The best characterized RAS effector pathway proceeds via a kinase cascade that involves phosphorylation of RAF. RAF in turn phosphorylates MEK which then phosphorylates ERK MAPKs. Activated ERKs translocate into the nucleus where they phosphorylate specific transcription factors that are involved in the regulation of various cellular responses, particularly in promoting cell proliferation.

Figure 3. The sonic hedgehog signalling pathway. When Shh is absent, the 12-pass transmembrane receptor protein ptch1 inhibits smoothened (Smo), a 7-pass G-protein-coupled-like receptor that acts as a transducer of the Shh signal. Smo is released upon binding of Shh to Ptch1 initiating a signal transduction cascade that results in activation of the multifunctional zinc finger transcription factors Gli. Gli proteins are sequestered in the cytoskeleton forming a complex with Fused and Sufu (suppressor of Fused). Activation of the pathway leads to dissociation of Gli from this complex and translocation into the nucleus. There are three human Gli proteins: Gli1, Gli2 and Gli3. Genes regulated by Shh include the Wnt family of growth factors, the bone morphogenetic proteins (BMPs) members of the TGF-β superfamily, the platelet-derived growth factor receptor PDGFRα, the winged helix domain transcription factor FOXE1 (which is specifically expressed in epidermal basal keratinocytes), as well as components of the Shh pathway itself, such as Ptch1, Ptch2 Gli1, and Gli2[9,10].

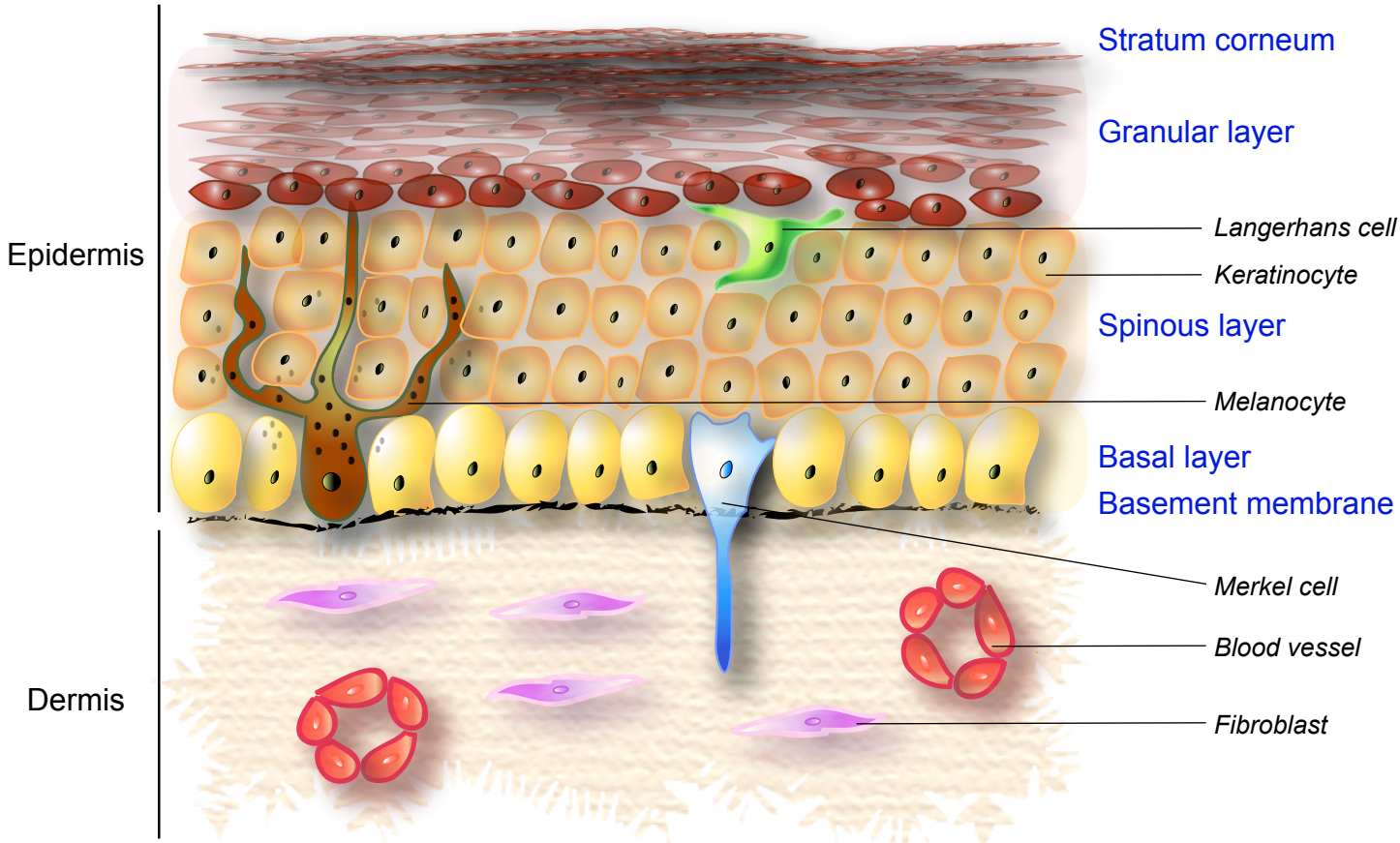
Figure 4. Genetic alterations involved in the development of malignant melanoma (A) and squamous cell carcinoma (B). Both cancers are thought to arise by a multi-step process. For additional details see the text. RGP, radial growth phase; VGP, vertical growth phase; AK, actinic keratoses; CIS, carcinoma *in situ*; SCC, squamous cell carcinoma.

Table 1. Frequency of mutations in components of the Shh pathway found in hereditary and sporadic BCCs*

	SHH	*PTCH1*	*SMO*
NBCC syndrome	-	Germline mutations and LOH	-
XP BCCs	18%	73-88%	30%
Sporadic BCCs	~1%	12-38%	6-13%

* Adapted from ref. 10

(A)


Epidermis
Dermis
Stratum corneum
Granular layer
Langerhans cell
Keratinocyte
Spinous layer
Melanocyte
Basal layer
Basement membrane
Merkel cell
Blood vessel
Fibroblast

(B)

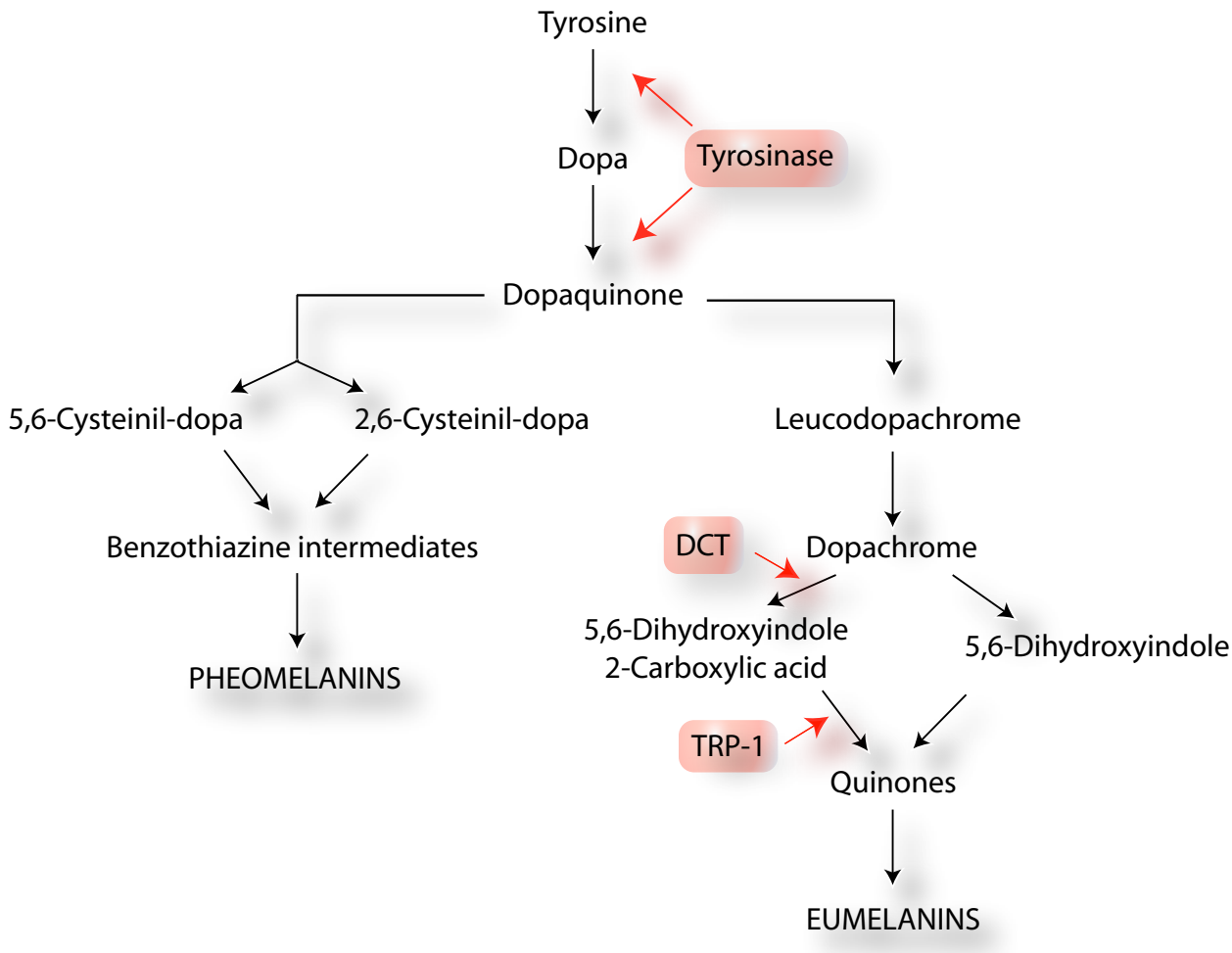
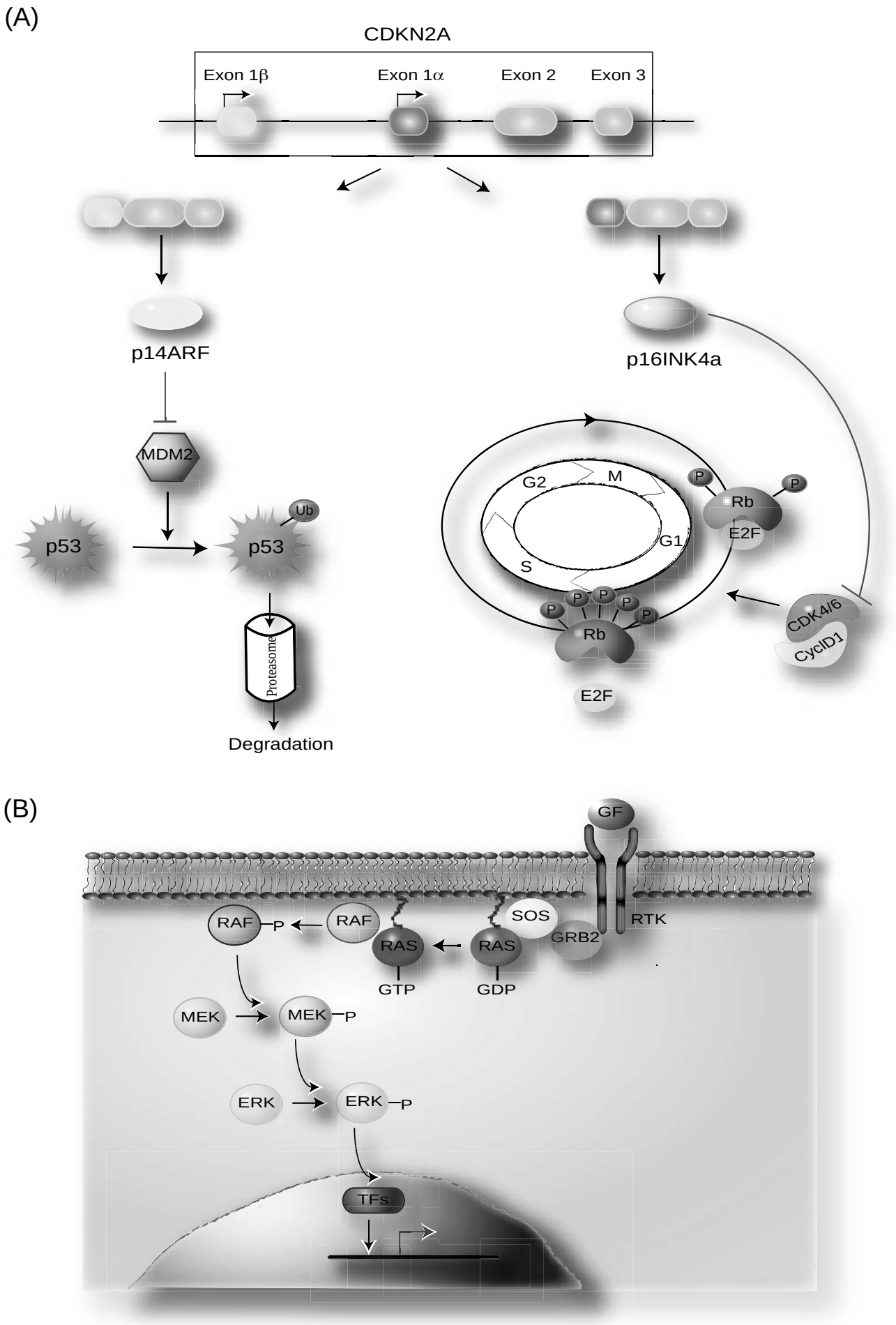

Tyrosine
Dopa
Tyrosinase
Dopaquinone
5,6-Cysteinil-dopa
2,6-Cysteinil-dopa
Leucodopachrome
Benzothiazine intermediates
DCT
Dopachrome
5,6-Dihydroxyindole
2-Carboxylic acid
5,6-Dihydroxyindole
PHEOMELANINS
TRP-1
Quinones
EUMELANINS

Figure 1


(A)
CDKN2A
Exon 1β
Exon 1α
Exon 2
Exon 3
p14ARF
p16INK4a
MDM2
p53
p53
Ub
Proteasome
Degradation
G2
M
G1
S
Rb
E2F
P
CDK4/6
CyclD1
(B)
GF
RTK
SOS
GRB2
RAS
RAS
GTP
GDP
RAF
RAF
P
MEK
MEK
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ERK
ERK
P
TFs

Figure 2

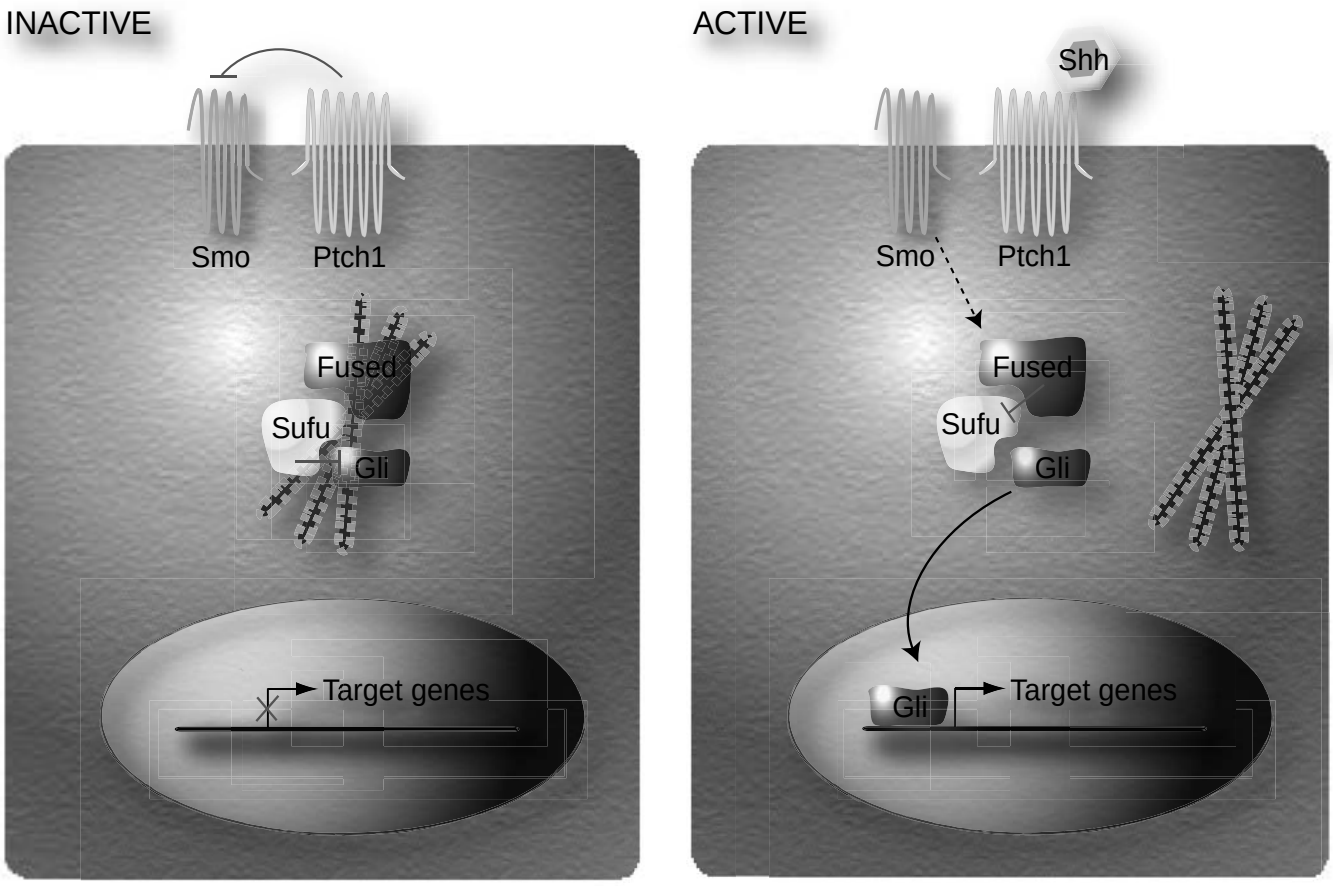
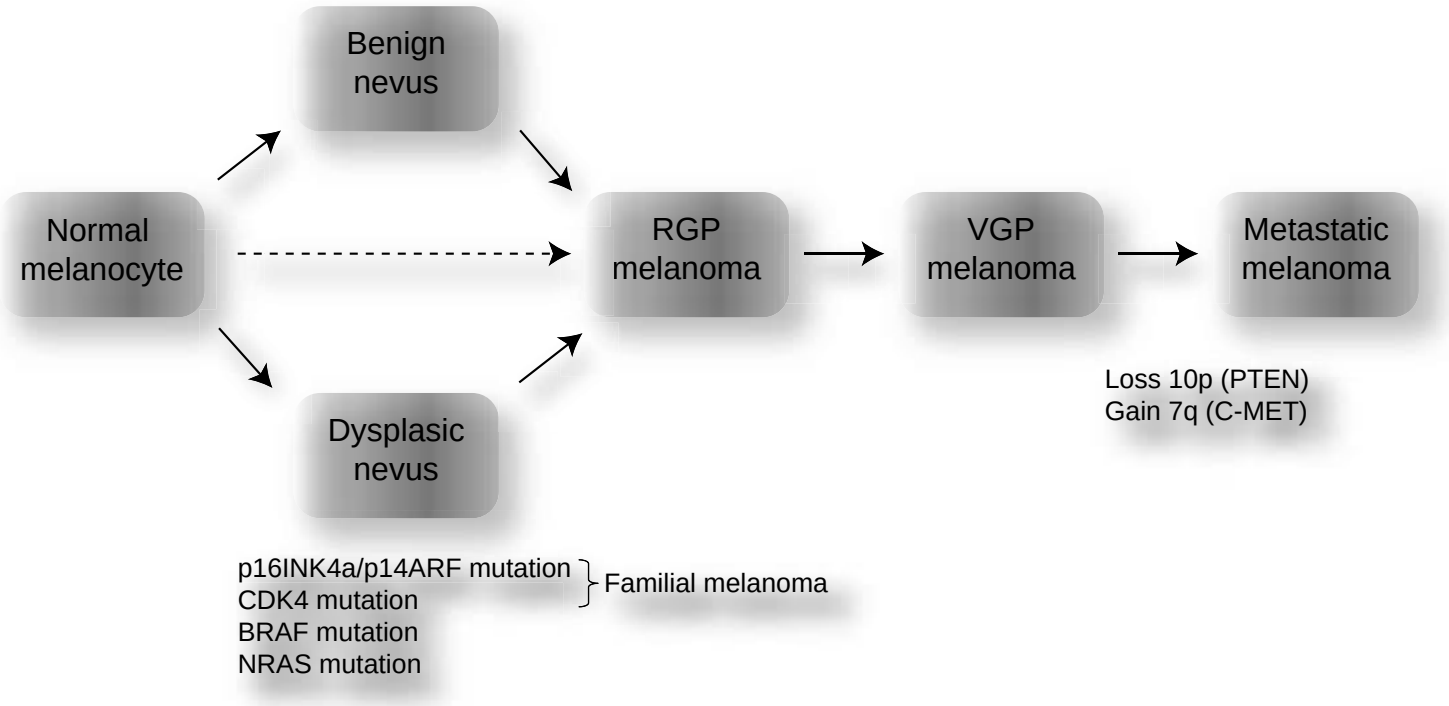

INACTIVE
Smo
Ptch1
Fused
Sufu
Gli
Target genes
ACTIVE
Shh
Smo
Ptch1
Fused
Sufu
Gli
Gli
Target genes

Figure 3

(A)


Benign nevus
Normal melanocyte
RGP melanoma
VGP melanoma
Metastatic melanoma
Loss 10p (PTEN)
Gain 7q (C-MET)
Dysplasic nevus
p16INK4a/p14ARF mutation
CDK4 mutation
BRAF mutation
NRAS mutation
Familial melanoma

(B)

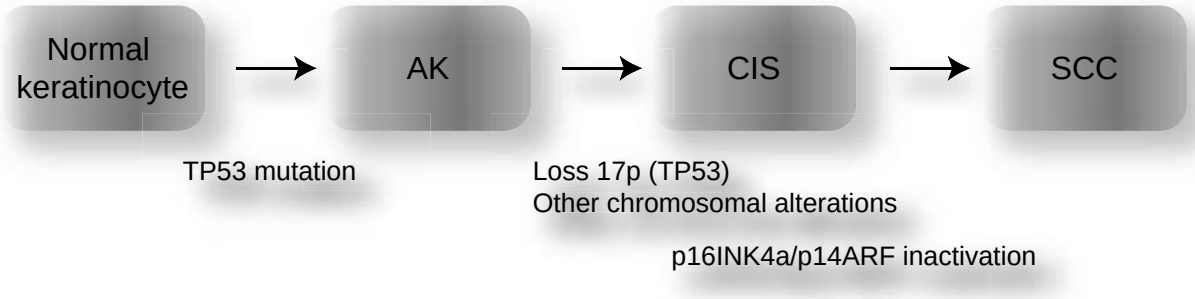

Normal keratinocyte
AK
CIS
SCC
TP53 mutation
Loss 17p (TP53)
Other chromosomal alterations
p16INK4a/p14ARF inactivation

Figure 4