

## REVIEW

# Molecular tumorigenesis of the skin

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**Abstract :** Skin tumors are supposed to develop through accumulations of genetic and/or epigenetic events in normal cells of the skin. Among them, we focus on common skin tumors, including benign, seborrheic keratosis, and malignant, squamous cell carcinoma and melanoma. Many important molecules have been detected on the molecular tumorigenesis of each of them to date, and some drugs targeted for their molecules have been already developed. We review updates on the molecular tumorigenesis of these tumors with our current works. *J. Med. Invest.* 61 : 7-14, February, 2014

**Keywords :** keratinocyte, melanocyte, seborrheic keratosis, squamous cell carcinoma, melanoma

## INTRODUCTION

The skin is a multilayered organ that exists in the outermost region of the body against the environments, and the outermost layer of the skin is the epidermis. In the epidermis, almost cells consist of epidermal keratinocytes, and some melanocytes scatter in the basal layer of the epidermis.

In this review, we focus on common skin tumors, including benign, seborrheic keratosis (SK), and malignant, squamous cell carcinoma (SCC) originated from keratinocytes, and malignant, melanoma originated from melanocytes. We describe updates on the molecular tumorigenesis of these tumors.

### 1. BENIGN SKIN TUMORS

#### *seborrheic keratosis (SK)*

SK is the most common benign skin tumor in the whole body, and more common in areas of sun

exposure, especially the face. According to results of transgenic mice, activation of fibroblast growth factor receptor (FGFR) 3 has been found to be relevant to the pathogenesis of SK (1). FGFR3 belongs to the FGFR family of transmembrane tyrosine kinase receptors, and binding of ligands to FGFR3 stimulates cell proliferation signals (2). The activation of FGFR3 through somatic point mutations has been identified in several kinds of cancer, e.g., bladder carcinoma, multiple myeloma, and cervical cancer (2). Transgenic mice with the expression of activated FGFR3 in the skin were reported in 2005 (1). They targeted FGFR3<sup>S249C</sup> (an activated mutant FGFR3) to the basal cells of the epidermis of transgenic mice. At the age of 3-4 months, the transgenic mice presented skin verrucous tumors on the eyelids and the snout, which showed similar histological features to human SK. In addition, they screened a series of 62 cases of SK for *FGFR3* mutations, and 24 cases of these tumors (39%) harbored somatic activated *FGFR3* mutations (1). We also examined mutations of *FGFR3* gene in the Japanese cases of SK, and activated *FGFR3* mutations were detected in 4 of 22 cases (18%) of SK (3). These activated mutations were same as those mutations found in cancers from the other organs in above.

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We examined FGFR3 protein expression by immunostainings because the above mutation rate in our cases was not high. Twenty-seven of 31 cases (87%) of SK showed moderately to strongly positive expression of FGFR3 protein, but almost cases of the other skin tumors, e.g., actinic keratosis, Bowen's disease, basal cell carcinoma, and SCC, were negative (3). Activation of FGFR3 might be a common feature on the tumorigenesis of SK regardless of the presence of the activated *FGFR3* mutations. Since activated *FGFR3* mutations were detected in flat lesions of SK and solar lentigo, activation of FGFR3 would be an early event on the development of SK (4, 5).

The transcriptional factor forkhead box N1 (FOXN1) has been identified as a downstream target of FGFR3 (6). Activation of FGFR3 induces the transcription of FOXN1, and FOXN1 also induces the transcription of FGFR3, indicating a positive feedback loop between FGFR3 and FOXN1 (6). The positive FGFR3/FOXN1 feedback loop in SK was confirmed by our analyses that all 11 cases of SK showed moderately to strongly positive expression of FOXN1 protein (7). FOXN1 might be also commonly activated in SK.

We have ever experienced a rare case of SCC developed in the lesion of SK. A 76-year-old female had a black plaque in her leg for over 30 years, and a red papule has developed in the lesion since 4 months (Fig. 1). The histological features showed spindle cell SCC in the lesion of SK (Fig. 2). Since the obvious expression of FGFR3 protein was observed in not spindle cell SCC but SK by immunostainings (Fig. 3), we supposed that down-regulation of FGFR3 expression might be associated with the development of spindle cell SCC in the lesion of SK.



Fig. 1 Clinical features.

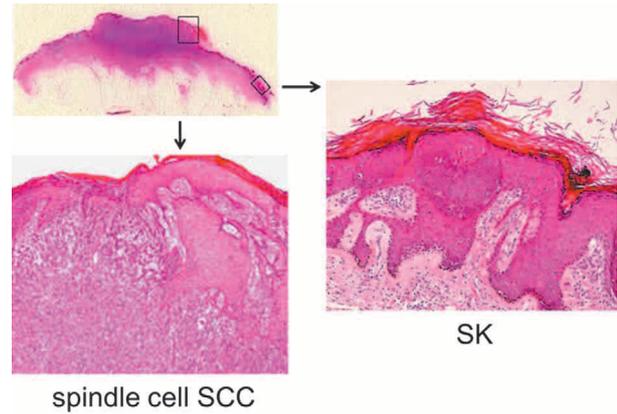


Fig. 2 Histological features.

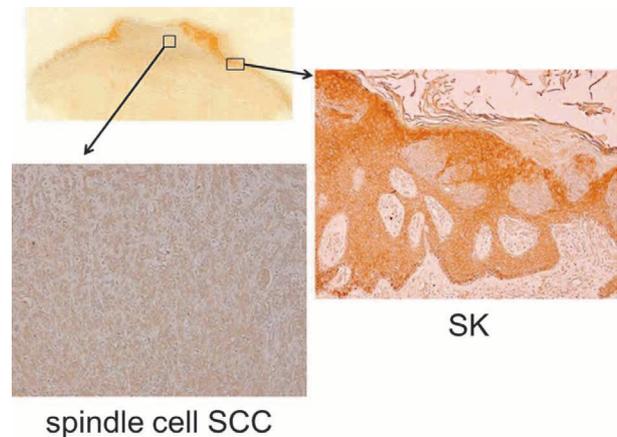


Fig. 3 Immunohistochemical stainings for FGFR3.

## 2. MALIGNANT SKIN TUMORS

It is widely accepted that normal keratinocytes or melanocytes in the epidermis can convert to SCC or melanoma, respectively, through the multistep process that involves activation of oncogenes and/or inactivation of tumor suppressor genes, similar to malignant tumors originated from the other organs. According to recent approaches by means of next-generation sequencing in SCC (8) and melanoma (9, 10), genomic DNA from tumor cells in a tumor was prove to be not homogeneous. Many mutations have been detected in genomic DNA from tumor cells in a tumor with a variety of mutant allele frequencies per each gene. A malignant tumor must be basically monoclonal, however, "intratumor heterogeneity" is commonly found in each of malignant tumors. Therefore, models of "clonal evolution (Fig. 4) (11)" and "trunk and branch (Fig. 5) (12)" have been proposed as a model representative of the development through the multistep process and a model representative of intratumor heterogeneity on the

tumorigenesis of malignant tumors, respectively.

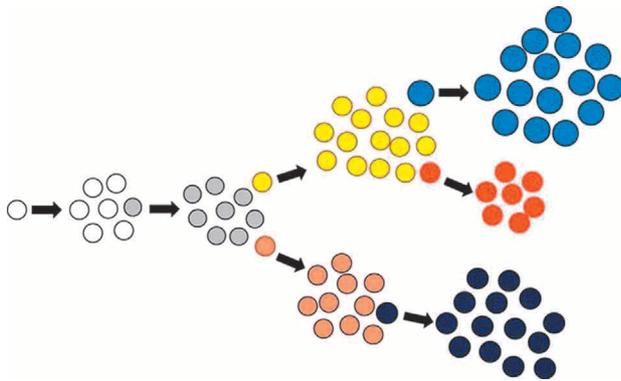


Fig. 4 Evolution of cancer cells.

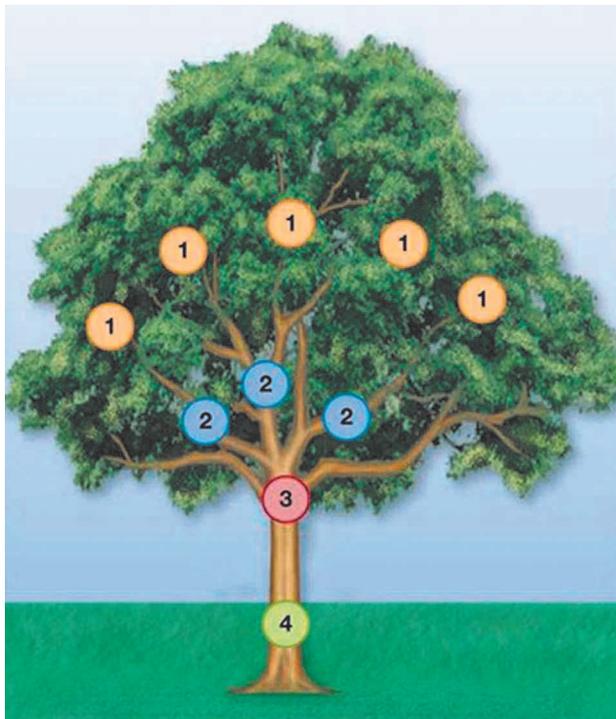


Fig. 5 Trunk and branch model. Common or ubiquitous events in the tumor found in every subclone and every tumor region are represented in the trunk of the tree. Diverse, heterogeneous somatic events are represented by the branches and the leaves.

### 1) squamous cell carcinoma (SCC)

SCC is one of the most common skin cancers associated with a substantial risk of metastasis (13). Exposure to the ultra-violet (UV), especially UVB, radiation is the most common cause of SCC in sunlight-exposed areas. If keratinocytes would fail to have DNA damages due to UVB repaired, the abnormalities of genes would remain in them. Hypercarcinogenic states for SCC, where keratinocytes

are susceptible to the occurrence and accumulation of gene mutations, consist of the inherited lesions, e.g., xeroderma pigmentosum that is defective in DNA base excision repair, and the acquired lesions, e.g., chronic ulcers, burn and posttraumatic scars. In addition, SCC occurs with high frequency in renal allograft recipients after prolonged immunosuppression, probably due to Human papillomavirus (HPV) infections, especially oncogenic types of HPV 16 and 18.

A novel approach by means of next-generation sequencing in SCC has been reported by a group of U.S.A. in 2011 (8). They performed exome-level sequencing of 8 cases of primary SCC and matched normal tissues, and detected a very large mutation burden of approximately 1,300 somatic single-nucleotide variants per SCC exome (1 per ~30,000 bp of coding sequence) (8). C>T transition base substitutions at dipyrimidine sites were the most common changes (> 85%), consistent with UV damage. Among 1,300 somatic mutations, almost all mutations would be “passenger” mutations (i.e., mutations that never conferred a fitness advantage), and some limited numbered mutations would be “driver” mutations (i.e., mutations that confer or at some point conferred a fitness advantage to the tumor cell). Although whether a mutation is a real driver mutation or not needs functional analyses with keratinocytes and/or model animals, they selected some candidates for driver genes based on many previous results as shown in Table 1 (8). Among them, we focus on well-known *TP53*, *CDKN2A*, and *HRAS* genes, and *NOTCH* genes as candidates for novel driver genes.

Inactivated mutations of *TP53* gene have been found in approximately half cases of SCC as well as various other human cancers (13-15). Allelic loss on 17p, where *TP53* gene locates, has been also observed in approximately from 20 to 40% of cases of SCC (16). As known as “guardian of the genome (17)”, the cells that *TP53* functions are lost would render resistant for cell growth arrest and apoptosis, and would be susceptible to the occurrence and accumulation of gene mutations in addition to accelerated cell growth. Because inactivated mutations of *TP53* gene have been found in lesions of solar keratosis and apparently histological normal skin, the mutations might occur at the early stage in the development of SCC (14). Seven of 8 cases of SCC also showed inactivation of both alleles of *TP53* gene as shown in Table 1 (8).

Similar to *TP53* gene, seven of 8 cases of SCC also

**Table 1.** Cases and selected mutations described in Table 1 (Ref. 8)

	Sex	Age	Site	Immune status	<i>TP53</i>	<i>CDKN2A</i>	<i>NOTCH1</i>	<i>NOTCH2</i>	<i>NOTCH3</i>	<i>NOTCH4</i>	Other known COSMIC mutations
1	M	76	Scalp	+	R248W	P135L	Q610X	W330X, R1838X			<i>EP300</i> , <i>WT1</i>
2	M	87	Scalp	+	E285K		P1771S, R1595Q		P226S		<i>WT1</i>
3	M	84	Left dorsal hand	+	E224 (Splice site)		C478F			R1333C	
4	F	61	Left cheek	+	Y220N					W309X	<i>PIK3CG</i>
5	M	83	Left cheek	+	H179Y, P278S		W1769X	Q1634X, T2278I		S1602F	
6	M	85	Right temple	+	P142N, H179Y	P133L	(Splice site)	S1836F, E297K			<i>EZH2</i>
7	M	58	Left aural helix	-	E286K, T329I, E349X		Q1924X	Q1616X, G488D			<i>HRAS</i>
8	M	63	Lower lip	+							<i>HSPB2</i>

Boldface denotes loss of wild-type allele.

showed inactivation of *NOTCH* genes as shown in Table 1 (8). Allelic loss on 9q, where *NOTCH1* gene locates, has been also observed in approximately from 20 to 40% of cases of SCC (16). *NOTCH1* mutations were detected in 9 of 11 cases of SCC (18), including 8 cases of SCC in Table 1 (8), and loss of normal allele of *NOTCH1* gene was found in 3 of 9 cases of SCC with inactivated *NOTCH1* mutations (18). Inactivated *NOTCH2*, *NOTCH3*, and *NOTCH4* mutations were detected in 5, 3, and 3 of 11 cases of SCC (18), including 8 cases of SCC in Table 1 (8), respectively. Since NOTCH has been reported to be a negative regulator of keratinocyte stem cell potential and inducer of differentiation (19), *NOTCH* genes must be novel driver genes.

*CDKN2A* gene encodes two different tumor suppressor gene products, p16<sup>INK4a</sup> and p14<sup>ARF</sup> (20). p16<sup>INK4a</sup> is involved in the function of cell growth suppression of RB1 by binding cyclin dependent kinase 4/6 (CDK4/6) and inhibiting their enzyme activities, and p14<sup>ARF</sup> is involved in the function of cell growth arrest and apoptosis of TP53 by binding MDM2 and stabilizing TP53. Since mutations of *CDKN2A* gene have been detected mainly in a common exon to both p16<sup>INK4a</sup> and p14<sup>ARF</sup>, their mutations would inactivate both of RB1 and TP53. Mutations of *CDKN2A* gene have been reported in up to 20% of SCCs (13), and allelic loss on 9p, where *CDKN2A* gene locates, has been also observed in approximately from 20 to 40% of cases of SCC (16). In addition, epigenetic abnormalities by promoter hypermethylation have been found in 35% of cases of SCC (21). Two of 8 cases of SCC also showed inactivation

of both alleles of *CDKN2A* gene as shown in Table 1 (8).

*HRAS* is one of three *RAS* genes, including *HRAS*, *KRAS*, and *NRAS*, and activated *RAS* mutations are one of the most common genetic abnormalities in various human cancers (22). The *RAS* proteins are small G-proteins, and transduce intracellular signals, especially mitogen-activated protein kinase (MAPK) cascades (RAS-RAF-MEK-ERK) and phosphatidylinositol-3 kinase (PI3K) cascades. Activated *RAS* mutations produce many tumor-promoting effects, e.g., accelerating cell growth, inhibiting apoptosis, through the activated downstream cascades. Activated *HRAS* mutations have not been frequently detected in cases of SCC. The rate of *HRAS* mutations was 5% in our cases of SCC (13), and one of 8 cases of SCC also showed an activated *HRAS* mutation as shown in Table 1 (8).

We have already performed functional analyses by transducing candidate driver genes in normal human keratinocytes (23, 24). Using high efficiency retroviral transductions in normal human primary keratinocytes, we expressed *HRAS*<sup>V12</sup> (an activated mutant *HRAS*), *CDK4*, *TP53*<sup>W248</sup> (a dominant-negative mutant TP53), and *TERT* either singly or in combination, and used these cells to regenerate human skin on SCID mice (23). Among them, a combination of *HRAS*<sup>V12</sup> and *CDK4* produced human skin tumors with histologic features of SCC at 6 weeks after grafting (23), indicating that *HRAS*<sup>V12</sup> and *CDK4* must be driver genes as capable of converting normal human epidermal tissue into invasive neoplasia. Tumor accelerating effects by *HRAS*<sup>V12</sup>

and CDK4 were summarized in Fig. 6. We believe that a combination of activation of RAS and inactivation of RB1/p16<sup>INK4a</sup> might be crucial to the carcinogenesis at least in a subset of cases of SCC, because we actually found a case of SCC with both an activated *HRAS* mutations and an inactivated *CDKN2A* mutation (Fig. 7) (13). In addition, the combination of *HRAS*<sup>V12</sup> and CDK4 produced invasive three-dimensional organotypic neoplasia from normal human epithelia from not only the skin, but also oropharynx, esophagus and cervix (25). Thus, the combination should be a common model of human SCC from the skin and the other organs.

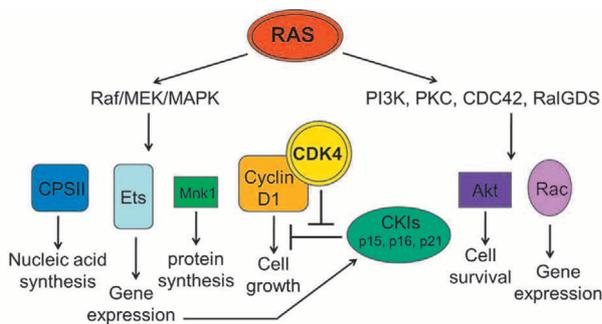


Fig. 6 Tumor accelerating effects by *HRAS*<sup>V12</sup> and CDK4.



Fig. 7 Clinical features. A reddish large tumor is seen in the parietal region of her head.

## 2) melanoma

melanoma is known to be the most aggressive malignant skin tumor. Most cases of melanoma in Japan show black because of tumors with large amounts of melanin. Much more reports about molecular tumorigenesis of melanoma have been accumulated so far, and several diver genes in melanoma have been already identified (26). We summarize

diver genes in melanoma in Fig. 8. Activation of *NRAS*, *BRAF*, and *KIT* genes was often observed as oncogenes, and *CDKN2A*, *PTEN*, and *TP53* genes were often inactivated as tumor suppressor genes (26). Both MAPK cascades and PI3 kinase cascades were activated through activation of *NRAS* gene, and MAPK cascades or PI3 kinase cascades were activated through activation of *BRAF* gene or inactivation of *PTEN* gene, respectively.

In terms of differences of the molecular tumorigenesis, melanoma was divided into 4 groups, including melanoma on skin without chronic sun-induced damage (non-CSD), melanoma on skin with chronic sun-induced damage (CSD), acral melanoma, and mucosal melanoma in 2006 (Fig. 9) (27). Acral melanoma is the most popular in Japan. As shown in Fig. 9, activated *BRAF* mutations were the most frequently observed, and activated *NRAS* mutations were relatively frequently observed in non-CSD melanoma. However, activated *KIT* mutations were relatively frequently observed in other 3 groups of melanoma. Among 3 groups of melanoma except for non-CSD melanoma, the frequencies of activated mutations of *BRAF* gene or *NRAS* gene varied.

Several approaches by means of next-generation sequencing have been already performed in melanoma (9, 10, 28). Activation of oncogenes and inactivation of tumor suppressor genes described in above have been confirmed, and some novel driver genes have been identified on the tumorigenesis of melanoma. Among them, some groups noticed *RAC1* as one of molecules associated with MAPK cascades (9, 10). Recurrent somatic activated *RAC1* mutations, inducing activation of MAPK cascades, were found in approximately 5% cases of melanoma. In addition to *RAC1*, many molecules associated with MAPK cascades were found to be activated in a subset of melanoma. Activation of MAPK cascades by activation of molecules, including *BRAF*, *NRAS*, and *RAC1*, was commonly observed in melanoma.

Same efforts as SCC for making human melanoma models have been performed in Stanford University (29). Similar to human SCC models, using high efficiency retroviral transductions in normal human primary melanocytes, they expressed candidate driver genes either singly or in combination, and used these cells with normal human keratinocytes to regenerate human skin on SCID mice. Human skin tumors with histologic features of melanoma were produced by three types of combinations of drivers: *NRAS*<sup>G12V</sup>, *CDK4*<sup>R24C</sup>, and *TERT*; *NRAS*<sup>G12V</sup>, *TP53*<sup>R248W</sup>, and *TERT*; *PI3K* p110 $\alpha$ , *CDK4*<sup>R24C</sup>,

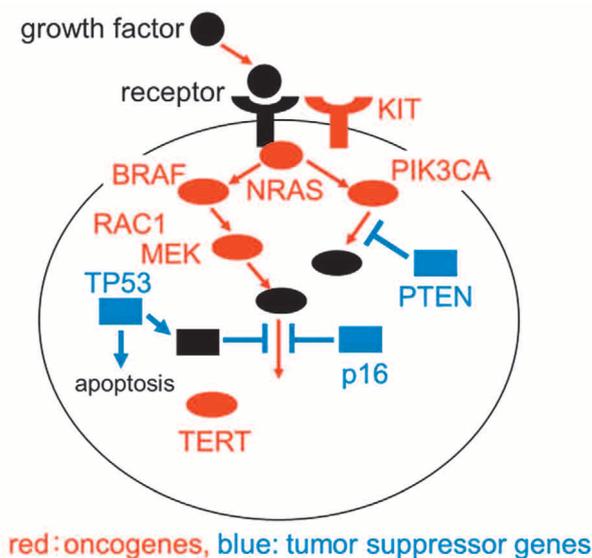


Fig. 8 Summary of driver genes in melanoma. A circle represents a melanoma cell.

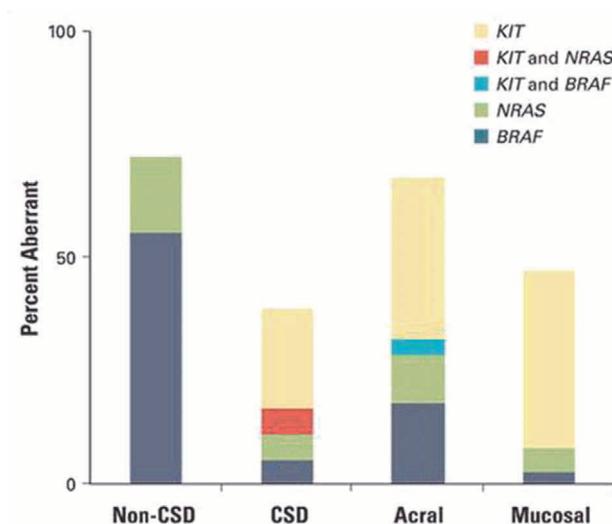


Fig. 9 Mutation frequencies in each of 4 groups of melanoma.

TP53<sup>R248W</sup>, and TERT (29). In contrast to human SCC models, TERT as a reverse transcriptase of telomerase was essential for human melanoma models.

Recently, activation of TERT has been reported in human melanoma (30, 31). Recurrent *TERT* promoter mutations were observed in 50 of 70 (71%) cases of melanoma, and the mutation increased transcriptional activities from the TERT promoter by 2 to 4-fold (30). Activation of TERT was commonly observed in melanoma (30, 31). These results were consistent with human melanoma models in above.

In addition to novel immunotherapies with anti-CTLA4 antibody and/or anti-PD-1 antibody, novel therapies based on the tumorigenesis of melanoma

have been tried for advanced melanoma (26, 32). Imatinib as an inhibitory drug to target KIT showed effective for a subset of melanoma with activated *KIT* mutations (33). Some inhibitory drugs to target BRAF or MEK showed effective for a subset of melanoma with activated *BRAF* mutations, and combination therapies of dual inhibition of BRAF and MEK showed more effective, although combination therapies were very expensive (32, 34). On the other hand, combination therapies of dual inhibition of MEK and CDK4 showed effective for a subset of melanoma with activated *NRAS* mutations (35). We believe that combination therapies of dual inhibition of MEK and CDK4 might be also effective for cases of SCC with activated *HRAS* mutations as shown in Fig. 6.

## CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

## CONCLUDING REMARKS

SCC and melanoma sometimes recur or metastasize after surgical excision. Advanced SCC and melanoma are often resistant for radiation treatment and chemotherapy. We hope that the molecular tumorigenesis of SCC and melanoma would be elucidated in detail to establish novel effective therapies for advanced SCC and melanoma based on their own molecular tumorigenesis.

## REFERENCES

- Logié A, Dunois-Lardé C, Rosty C, Levrel O, Blanche M, Ribeiro A, Gasc JM, Jorcano J, Werner S, Sastre-Garau X, Thiery JP, Radvanyi F : Activating mutations of the tyrosine kinase receptor FGFR3 are associated with benign skin tumors in mice and humans. *Hum Mol Genet* 14 : 1153, 2005
- L'Hôte CG, Knowles MA : Cell responses to FGFR3 signalling : growth, differentiation and apoptosis. *Exp Cell Res* 304 : 417-431, 2005
- Hida Y, Kubo Y, Arase S : Activation of fibroblast growth factor receptor 3 and oncogene-induced senescence in skin tumours. *Br J Dermatol* 160 : 1258-1263, 2009

4. Hafner C, Hartmann A, van Oers JM, Stoehr R, Zwarthoff EC, Hofstaedter F, Landthaler M, Vogt T : FGFR3 mutations in seborrheic keratoses are already present in flat lesions and associated with age and localization. *Mod Pathol* 20 : 895-903, 2007
5. Hafner C, Stoehr R, van Oers JM, Zwarthoff EC, Hofstaedter F, Landthaler M, Hartmann A, Vogt T : FGFR3 and PIK3CA mutations are involved in the molecular pathogenesis of solar lentigo. *Br J Dermatol* 160 : 546-551, 2009
6. Mandinova A, Mandinova A, Kolev V, Neel V, Hu B, Stonely W, Lieb J, Wu X, Colli C, Han R, Pazin MJ, Ostano P, Dummer R, Brissette JL, Dotto GP : A positive FGFR3/FOXN1 feedback loop underlies benign skin keratosis versus squamous cell carcinoma formation in humans. *J Clin Invest* 119 : 3127-3137, 2009
7. Ishigami T, Hida Y, Matsudate Y, Murao K, Kubo Y : The involvement of fibroblast growth factor receptor signaling pathways in dermatofibroma and dermatofibrosarcoma protuberans. *J Med Invest* 60 : 256-261, 2013
8. Durinck S, Ho C, Wang NJ, Liao W, Jakkula LR, Collisson EA, Pons J, Chan SW, Lam ET, Chu C, Park K, Hong SW, Hur JS, Huh N, Neuhaus IM, Yu SS, Grekin RC, Mauro TM, Cleaver JE, Kwok PY, LeBoit PE, Getz G, Cibulskis K, Aster JC, Huang H, Purdom E, Li J, Bolund L, Arron ST, Gray JW, Spellman PT, Cho RJ : Temporal dissection of tumorigenesis in primary cancers. *Cancer Discov* 1 : 137-143, 2011
9. Krauthammer M, Kong Y, Ha BH, Evans P, Bacchiocchi A, McCusker JP, Cheng E, Davis MJ, Goh G, Choi M, Ariyan S, Narayan D, Dutton-Regester K, Capatana A, Holman EC, Bosenberg M, Sznol M, Kluger HM, Brash DE, Stern DF, Materin MA, Lo RS, Mane S, Ma S, Kidd KK, Hayward NK, Lifton RP, Schlessinger J, Boggon TJ, Halaban R : Exome sequencing identifies recurrent somatic RAC1 mutations in melanoma. *Nat Genet* 44 : 1006-1014, 2012
10. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, Nickerson E, Auclair D, Li L, Place C, Dicara D, Ramos AH, Lawrence MS, Cibulskis K, Sivachenko A, Voet D, Saksena G, Stransky N, Onofrio RC, Winckler W, Ardlie K, Wagle N, Wargo J, Chong K, Morton DL, Stemke-Hale K, Chen G, Noble M, Meyerson M, Ladbury JE, Davies MA, Gershenwald JE, Wagner SN, Hoon DS, Schadendorf D, Lander ES, Gabriel SB, Getz G, Garraway LA, Chin L : A landscape of driver mutations in melanoma. *Cell* 150 : 251-263, 2012
11. Greaves M, Maley CC : Clonal evolution in cancer. *Nature* 481 : 306-313, 2012
12. Swanton C : Intratumor heterogeneity : evolution through space and time. *Cancer Res* 72 : 4875-4882, 2012
13. Kubo Y, Murao K, Matsumoto K, Arase S : Molecular carcinogenesis of squamous cell carcinomas of the skin. *J Med Invest* 49 : 111-117, 2002
14. Brash DE : Roles of the transcription factor p53 in keratinocyte carcinomas. *Br J Dermatol* 154 Suppl 1 : 8-10, 2006
15. Soussi T : TP53 mutations in human cancer : database reassessment and prospects for the next decade. *Adv Cancer Res* 110 : 107-139, 2011
16. Ashton KJ, Weinstein SR, Maguire DJ, Griffiths LR : Chromosomal aberrations in squamous cell carcinoma and solar keratoses revealed by comparative genomic hybridization. *Arch Dermatol* 139 : 876-882, 2003
17. Efeyan A, Serrano M : p53 : guardian of the genome and policeman of the oncogenes. *Cell Cycle* 6 : 1006-1010, 2007
18. Wang NJ, Sanborn Z, Arnett KL, Bayston LJ, Liao W, Proby CM, Leigh IM, Collisson EA, Gordon PB, Jakkula L, Pennypacker S, Zou Y, Sharma M, North JP, Vemula SS, Mauro TM, Neuhaus IM, Leboit PE, Hur JS, Park K, Huh N, Kwok PY, Arron ST, Massion PP, Bale AE, Haussler D, Cleaver JE, Gray JW, Spellman PT, South AP, Aster JC, Blacklow SC, Cho RJ : Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. *Proc Natl Acad Sci USA* 108 : 17761-17766, 2011
19. Dotto GP : Notch tumor suppressor function. *Oncogene* 27 : 5115-5123, 2008
20. Gil J, Peters G : Regulation of the INK4b-ARF-INK4a tumour suppressor locus : all for one or one for all. *Nat Rev Mol Cell Biol* 7 : 667-677, 2006
21. Murao K, Kubo Y, Ohtani N, Hara E, Arase S : Epigenetic abnormalities in cutaneous squamous cell carcinomas : frequent inactivation of the RB1/p16 and p53 pathways. *Br J Dermatol* 155 : 999-1005, 2006

22. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D : RAS oncogenes : weaving a tumorigenic web. *Nat Rev Cancer* 11 : 761-774, 2011
23. Lazarov M, Kubo Y, Cai T, Dajee M, Tarutani M, Lin Q, Fang M, Tao S, Green CL, Khavari PA : CDK4 coexpression with Ras generates malignant human epidermal tumorigenesis. *Nat Med* 8 : 1105-1114, 2002
24. Dajee M, Lazarov M, Zhang JY, Cai T, Green CL, Russell AJ, Marinkovich MP, Tao S, Lin Q, Kubo Y, Khavari PA : NF-kappaB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. *Nature* 421 : 639-643, 2003
25. Ridky TW, Chow JM, Wong DJ, Khavari PA : Invasive three-dimensional organotypic neoplasia from multiple normal human epithelia. *Nat Med* 16 : 1450-1455, 2010
26. Eggermont AM, Spatz A, Robert C : Cutaneous melanoma. *Lancet* S0140-6736(13)60802-8, 2013
27. Curtin JA, Busam K, Pinkel D, Bastian BC : Somatic activation of KIT in distinct subtypes of melanoma. *J Clin Oncol* 24 : 4340-4346, 2006
28. Kunz M, Dannemann M, Kelso J : High-throughput sequencing of the melanoma genome. *Exp Dermatol* 22 : 10-17, 2013
29. Chudnovsky Y, Adams AE, Robbins PB, Lin Q, Khavari PA : Use of human tissue to assess the oncogenic activity of melanoma-associated mutations. *Nat Genet* 37 : 745-749, 2005
30. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA : Highly recurrent TERT promoter mutations in human melanoma. *Science* 339 : 957-959, 2013
31. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, Hemminki K, Schadendorf D, Kumar R : TERT promoter mutations in familial and sporadic melanoma. *Science* 339 : 959-961, 2013
32. Garber K : Melanoma combination therapies ward off tumor resistance. *Nat Biotechnol* 31 : 666-668, 2013
33. Hodi FS, Corless CL, Giobbie-Hurder A, Fletcher JA, Zhu M, Marino-Enriquez A, Friedlander P, Gonzalez R, Weber JS, Gajewski TF, O'Day SJ, Kim KB, Lawrence D, Flaherty KT, Luke JJ, Collichio FA, Ernstoff MS, Heinrich MC, Beadling C, Zukotynski KA, Yap JT, Van den Abbeele AD, Demetri GD, Fisher DE : Imatinib for melanomas harboring mutationally activated or amplified KIT arising on mucosal, acral, and chronically sun-damaged skin. *J Clin Oncol* 31 : 3182-3190, 2013
34. Jang S, Atkins MB : Which drug, and when, for patients with BRAF-mutant melanoma? *Lancet oncol* 14 : e60-69, 2013
35. Kwong LN, Costello JC, Liu H, Jiang S, Helms TL, Langsdorf AE, Jakubosky D, Genovese G, Muller FL, Jeong JH, Bender RP, Chu GC, Flaherty KT, Wargo JA, Collins JJ, Chin L : Oncogenic NRAS signaling differentially regulates survival and proliferation in melanoma. *Nat Med* 18 : 1503-1510, 2012