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 $FGFR3^{S249C}$ (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.

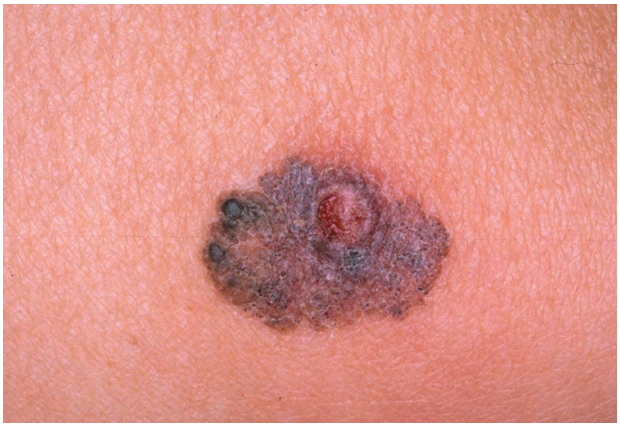

Fig. 1 Clinical features.

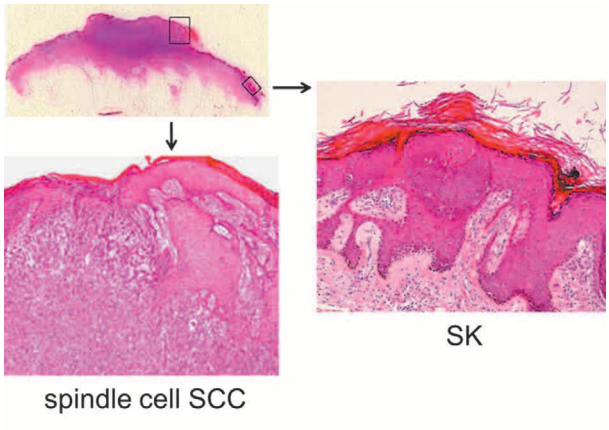

Fig. 2 Histological features.

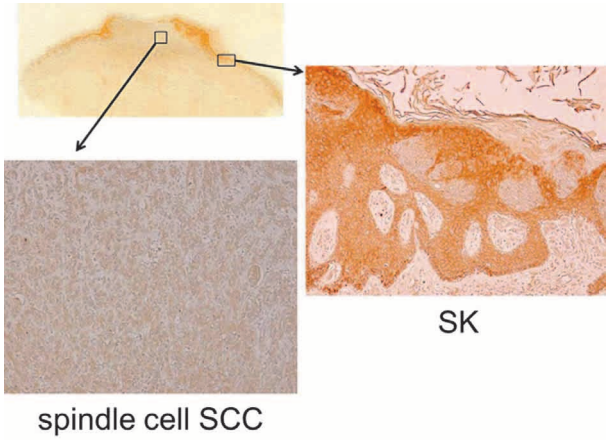

Fig. 3 Immunohistochemical stainings for FGFR3.

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.

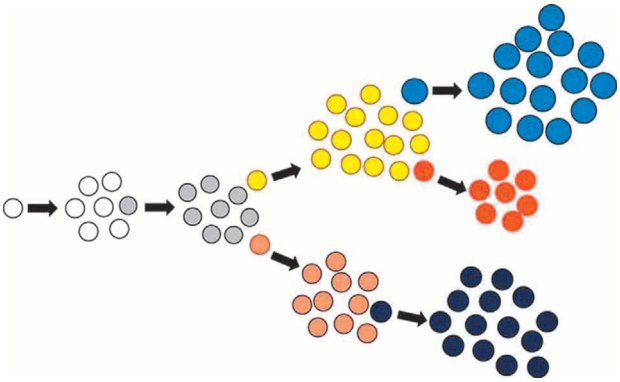

Fig. 4 Evolution of cancer cells.

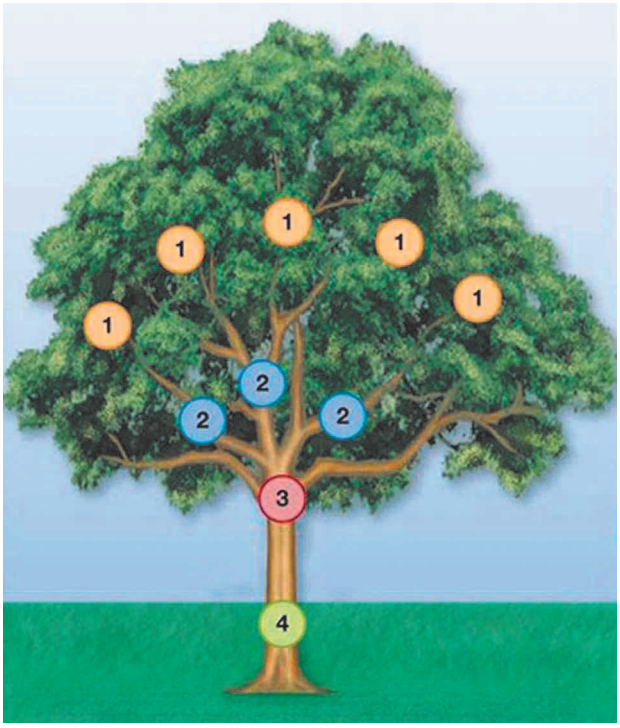

Fig. 5 Trunk and branch model. Common or ubiquitous events in the tumor found in every subclone and every tumor region are represented in the trunk of the tree. Diverse, heterogeneous somatic events are represented by the branches and the leaves.

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

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, $p16^{INK4a}$ and $p14^{ARF}$ (20). $p16^{INK4a}$ 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 $p14^{ARF}$ 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 $p16^{INK4a}$ and $p14^{ARF}$, 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 $HRAS^{V12}$ (an activated mutant HRAS), CDK4, $TP53^{W248}$ (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 $HRAS^{V12}$ and CDK4 produced human skin tumors with histologic features of SCC at 6 weeks after grafting (23), indicating that $HRAS^{V12}$ and CDK4 must be driver genes as capable of converting normal human epidermal tissue into invasive neoplasia. Tumor accelerating effects by $HRAS^{V12}$

and CDK4 were summarized in Fig. 6. We believe that a combination of activation of RAS and inactivation of RB1/p16^{INK4a} 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^{V12} 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.

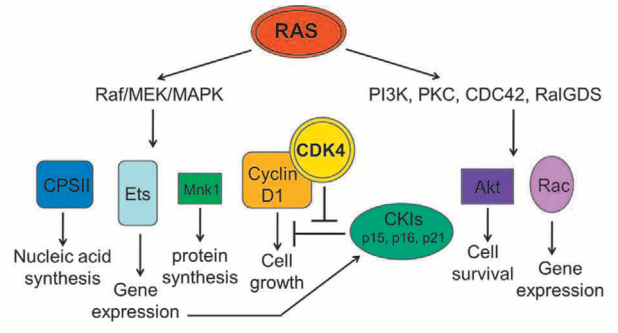

Fig. 6 Tumor accelerating effects by HRAS^{V12} and CDK4.

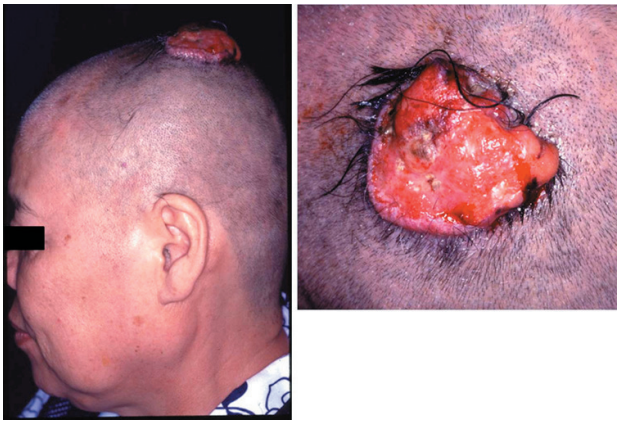

Fig. 7 Clinical features. A reddish large tumor is seen in the parietal region of her head.

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 chromic sun-induced damage (non-CSD), melanoma on skin with chromic 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^{G12V}, CDK4^{R24C}, and TERT ; NRAS^{G12V}, TP53^{R248W}, and TERT ; PI3K p110α, CDK4^{R24C},

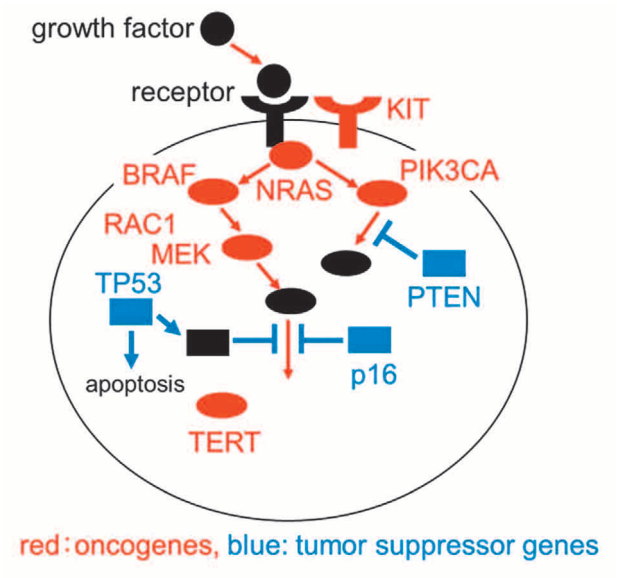

Fig. 8 Summary of driver genes in melanoma. A circle represents a melanoma cell.

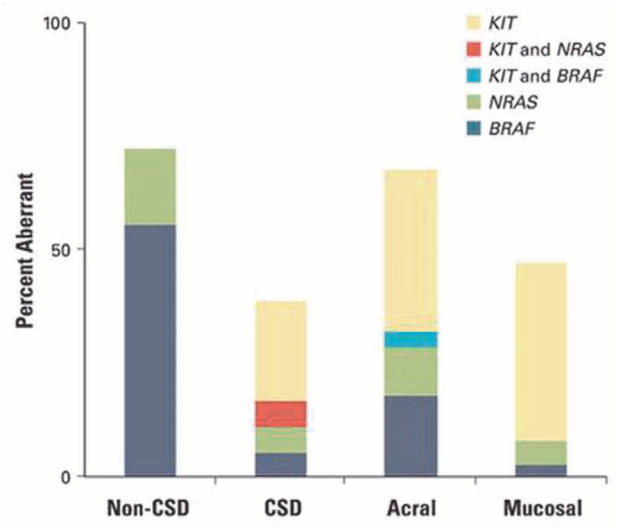

Fig. 9 Mutation frequencies in each of 4 groups of melanoma.

$TP53^{R248W}$, 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.

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