Analysis of the expression of a transcription factor, E2F4, in cutaneous squamous cell carcinoma (SCC)

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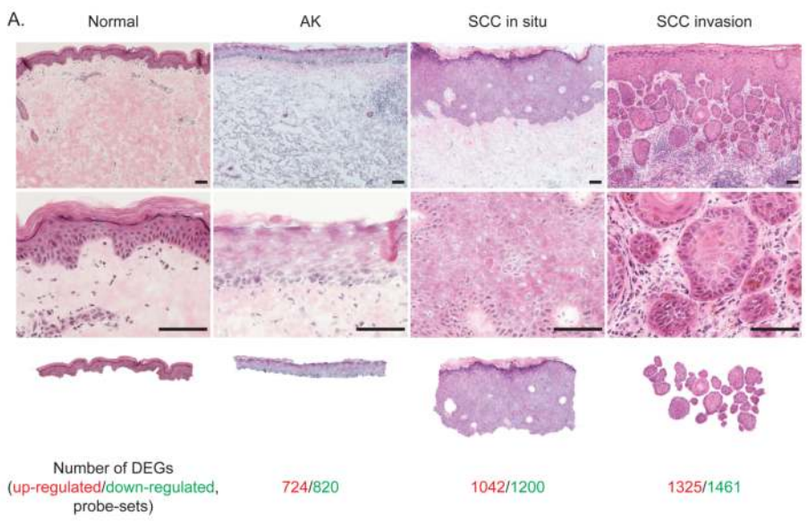
ABSTRACT

There is an increasing trend to treat specific cancers with cell-specific or pathway-specific antagonists. Although second most frequent skin cancer, the molecular mechanisms underlying development and progression of cutaneous SCC remains unclear. We has been studying SCC on the basis of differentially expressed gene list generated by the combination of laser capture microdissection (LCM) and cDNA microarray technology (Fig 1). One transcription factor that was significantly upregulated in actinic keratosis, a known pre-cancerous condition, and SCC compared to normal epidermis was E2F4 (FCH>15 and FDR<10^{-4}). E2F4 is a member of the E2F family of transcription factors that have a critical role in the control of cellular proliferation and apoptosis. Recent studies identified the roles of E2F4 in cancer, including breast carcinoma, prostate cancer, and melanoma. However, its roles in SCC have not been studied to date. In this study, we aimed to elucidate the functions of E2F4 in development and progression of cutaneous SCC. To achieve this, we first evaluated and compared the expression of E2F4 in various skin conditions. The specific expression of E2F4 in the nucleus of SCC tissues but not in other skin conditions, such as basal cell carcinoma (BCC), seborrheic keratosis, and psoriasis was identified by immunohistochemistry. We further confirmed that SCC cell lines (A431 and HSC-5) expressed E2F4 at genomic and protein levels. Our results suggest that E2F4 may have some functions in development and progression of cutaneous SCC.

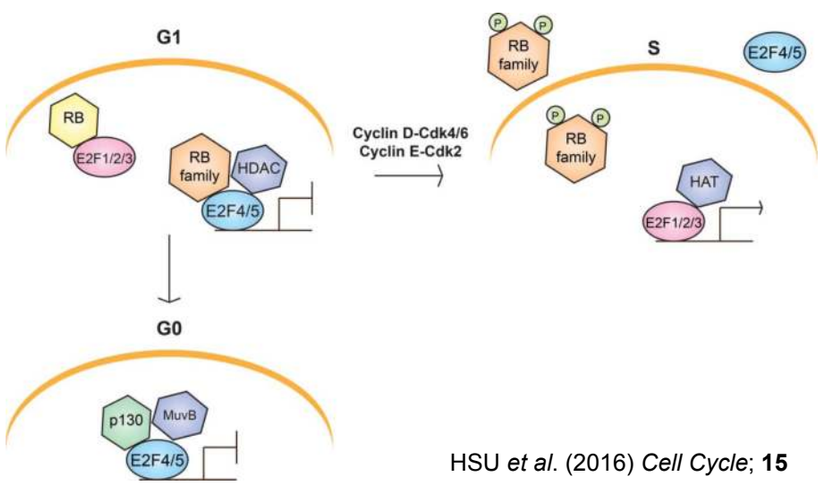


Affymetrix HGU133 A2.0, FCH>3.0, FDR<0.05

Mitsui *et al*. (2014) *JID*; **134**

Fig 1) An image of laser capture microdissection.
LCM was performed to collect epidermal regions from various skin tissues. AK: actinic keratosis

BACKGROUND



HSU *et al*. (2016) *Cell Cycle*; **15**

Fig 2) A scheme of the RB/E2F pathway in cell cycle progression.

The E2F family of transcription factors is a key determinant of cell proliferation in response to extra- and intra-cellular signals.

E2F4 is known as a transcriptional repressor, critical to engage and maintain cell cycle arrest in G0/G1 in conjunction with the retinoblastoma (RB) family proteins, i.e. pRb, p107, and p130.

Recent studies suggested new roles of E2F4 as an oncogene in several cancers, such as prostate and breast carcinomas (Waghray *et al*. (2001) *Cancer Res*; **61**, Rakha *et al*. (2004) *J Pathol*; **203**, Rakha *et al*. (2005) *Int J Cancer*; **114**).

The functions of E2F4 in the development and progression in cutaneous SCC have not been identified.

RESULTS

	AK		SCC in situ		invasive SCC	
Symbol	FCH	FDR	FCH	FDR	FCH	FDR
E2F4	15.49	<10^{-4}	15.50	<10^{-4}	16.08	<10^{-4}
RUNX1	10.47	<10^{-4}	18.69	<10^{-4}	24.26	<10^{-4}
PITX1	8.36	<10^{-4}	11.90	<10^{-4}	15.25	<10^{-4}
SPDEF	7.95	<10^{-4}	7.69	<10^{-4}	7.40	<10^{-4}
NR4A3	6.87	<10^{-4}	4.68	<10^{-4}	7.03	<10^{-4}
PAX8	6.84	<10^{-4}	5.93	<10^{-4}	8.54	<10^{-4}
SPDEF	6.74	<10^{-4}	6.70	<10^{-4}	6.49	<10^{-4}
NR4A1	5.87	<10^{-4}	2.56	0.01	3.25	<10^{-4}
RELB	5.50	<10^{-4}	3.50	<10^{-4}	6.08	<10^{-4}
FOXE1	5.41	<10^{-4}	9.97	<10^{-4}	5.20	<10^{-4}

Table 1) Transcription factors (TFs) upregulated in the various epidermal regions compared to normal epidermis.
E2F4 was the most highly expressed TF gene in the AK region based on cDNA microarray analysis. FCH: fold change compared to normal epidermis, FDR: false discovery rate.

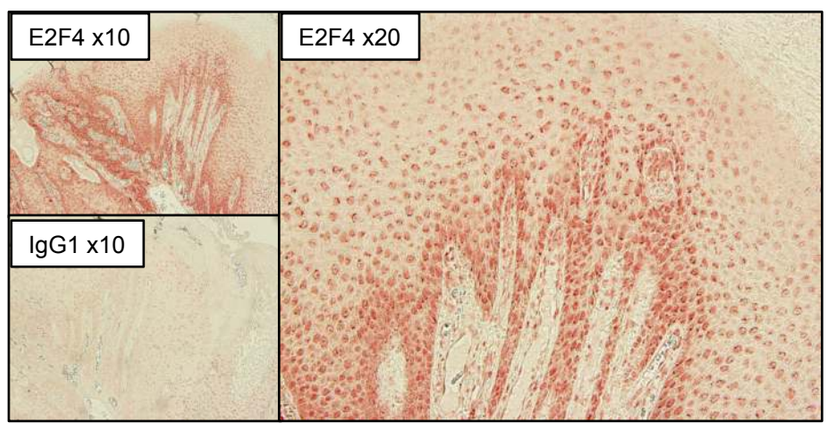


Fig 2) Expression of E2F4 in the SCC tissue.
E2F4 antibody stained the nucleus of the SCC tissues whereas control IgG1 antibody did not.

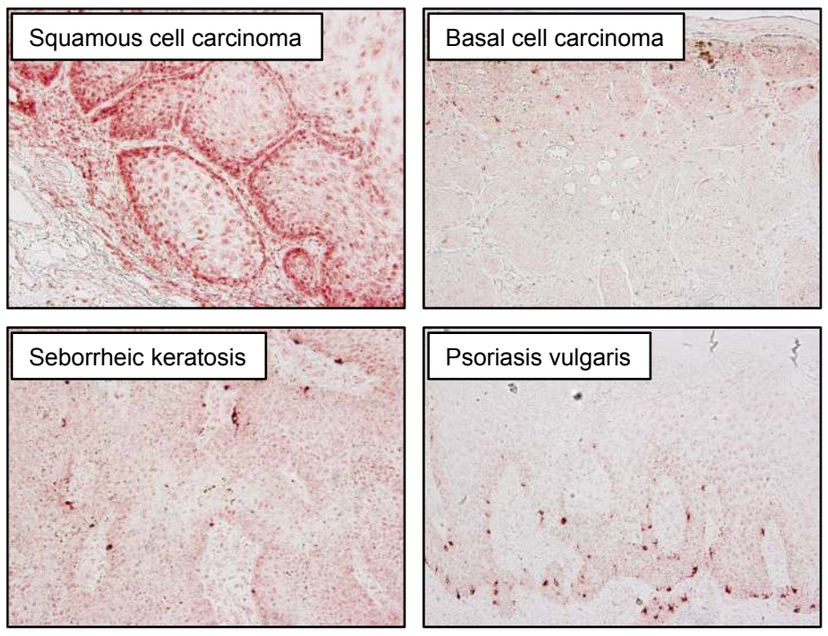


Fig 3) SCC specific expression of E2F4 among various skin conditions.
Expression of E2F4 was evaluated in the various skin conditions. E2F4 antibody stained SCC tissues (n=5) , whereas it was absent in the BCC (n=3), seborrheic keratosis (n=3), and psoriasis vulgaris (n=3). The E2F4 positive cells in BCC, seborrheic keratosis, and psoriasis vulgaris were melanocytes, an internal positive control.

MATERIALS & METHODS

Archived FFPE tissue of SCC (n=5), BCC (n=5), seborrheic keratosis (n=3), and psoriasis vulgaris (n=3) were used for immunohistochemistry.

A431 cells and HSC-5 cells were purchased from JCRB Cell Bank.

Antibodies used in this study were as follows;
E2F4 mAb; clone 4E2F04
E2F4 mAb; clone SPM179

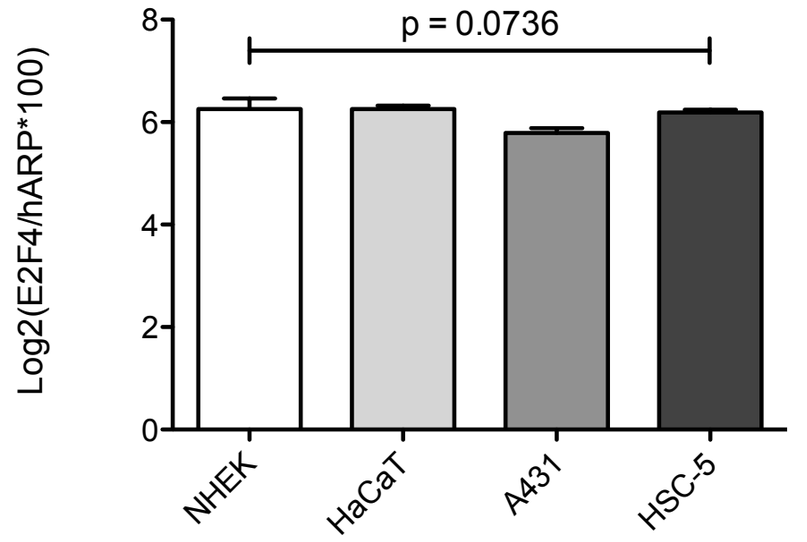


Fig 4) mRNA expression of E2F4 in the SCC cell lines.
mRNA expression of E2F4 was examined in the various keratinocytic cell lines by means of quantitative RT-PCR. E2F4 mRNA was expressed in the proliferating keratinocytic cells. NHEK: normal human epidermal keratinocute, HSC-5: a cutaneous SCC cell line established from a male Japanese patient

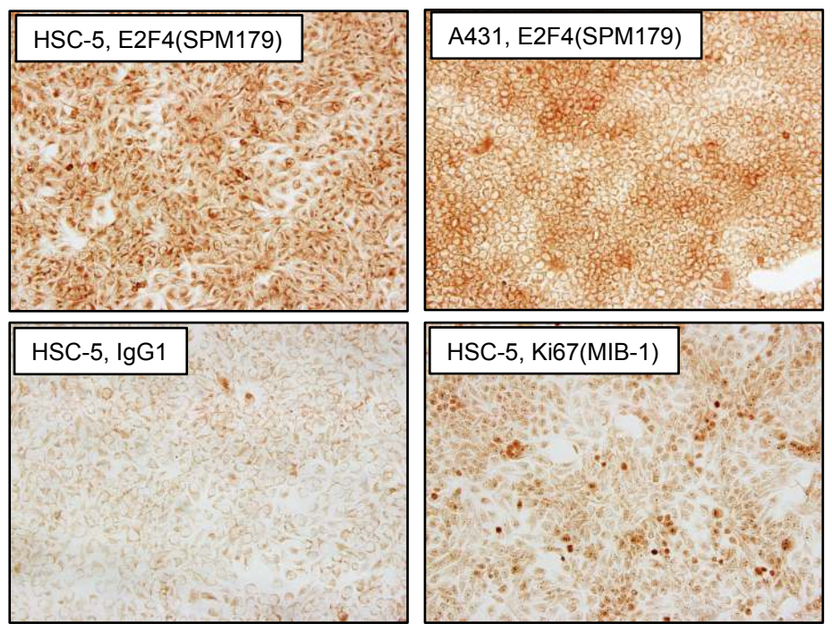


Fig 5) Protein expression of E2F4 in the SCC cell lines.
Protein expression of E2F4 was examined in the SCC cell lines by immunocytechemistry. E2F4 antibody stained the nucleus of highly proliferating HSC-5 cells, whereas it was positive in the cytoplasm of the A431 cells.

SUMMARY

E2F4 was identified as the most upregulated TF in the AK region by cDNA microarray analysis.

E2F4 protein was expressed in the nucleus of human SCC tissues.

E2F4 was expressed in the SCC cell lines at mRNA and protein levels.

CONCLUSION

E2F4 may have some functions in the development and progression of cutaneous SCC, though functional analyses will be required.

REFFERNCES

Hsu *et al*. (2016) *Cell Cycle*; **15**

Mitsui *et al*. (2014) *J Invest Dermatol*; **134**

Rakha *et al*. (2005) *Int J Cancer*; **114**

Rakha *et al*. (2004) *J Pathol*; **203**

Waghray *et al*. (2001) *Cancer Res*; **61**