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Constitutive transgene expression of Stem Cell Antigen-1 in the hair follicle alters the sensitivity to tumor formation and progression

Rikke Christensen [a,c], David M. Owens [b,f], Annette C. Füchtbauer [d], Anders Gunnarsson [a,c], Mette Ramsing [e], Ernst-Martin Füchtbauer [d], Uffe Birk Jensen [a,*]

[a] Department of Clinical Genetics, Aarhus University Hospital, Brendstrupgaardsvej 21C, 8200 Aarhus N, Denmark
[b] Department of Dermatology, Columbia University Medical Center, 1150 St. Nicholas Avenue, New York, NY 10032, USA
[c] Department of Biomedicine, Aarhus University, Bartholins Allé 6, 8000 Aarhus C, Denmark
[d] Department of Molecular Biology and Genetics, Aarhus University, C. F. Møllers Allé 3, 8000 Aarhus C, Denmark
[e] Department of Pathology, Aarhus University Hospital, Palle Juul- Jensens Boulevard 99, 8200 Aarhus N, Denmark
[f] Department of Pathology & Cell Biology, Columbia University Medical Center, 1150 St. Nicholas Avenue, New York, NY 10032, USA

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ABSTRACT

The cell surface protein Stem Cell Antigen-1 (Sca-1) marks stem or progenitor cells in several murine tissues and is normally upregulated during cancer development. Although the specific function of Sca-1 remains unknown, Sca-1 seems to play a role in proliferation, differentiation and cell migration in a number of tissues. In the skin epithelium, Sca-1 is highly expressed in the interfollicular epidermis but is absent in most compartments of the hair follicle; however, the function of Sca-1 in the skin has not been investigated. To explore the role of Sca-1 in normal and malignant skin development we generated transgenic mice that express Sca-1 in the hair follicle stem cells that are normally Sca-1 negative. Development of hair follicles and interfollicular epidermis appeared normal in Sca-1 mutant mice; however, follicular induction of Sca-1 expression in bulge region and isthmus stem cells reduced the overall yield of papillomas in a chemical carcinogenesis protocol. Despite that fewer papillomas developed in transgenic mice a higher proportion of the papillomas underwent malignant conversion. These findings suggest that overexpression of Sca-1 in the hair follicle stem cells contributes at different stages of tumour development. In early stages, overexpression of Sca-1 decreases tumour formation while at later stages overexpression of Sca-1 seems to drive tumours towards malignant progression.

1. Introduction

The epidermis is a multilayered epithelium that provides a protective cover of the external body surface. It consists of the interfollicular epidermis (IFE), the hair follicle (HF) and the sebaceous gland (SG). A complex hierarchy of distinct progenitor cell populations resides in the epidermis in order to maintain a functional epidermis during normal homeostasis (Page et al., 2013). The progenitor cells in the various compartments can be identified by the expression of subsets of different molecular markers. The bulge region stem cells of the hair follicle express CD34 and keratin 15 (Blanpain et al., 2004; Lyle et al., 1998; Trempus et al., 2003). Partly overlapping populations of the progenitor cells in the lower part of the bulge region and hair germ express Lgr5 (Jaks et al., 2008) and Gli1 (Brownell et al., 2011) respectively. The isthmus and upper isthmus contains multiple partly overlapping populations marked by the expression of Lgr6, Plet1/Mts24, and Lrig1 (Jensen et al., 2009; Nijhof et al., 2006; Snippert et al., 2010). Upon wounding, all progenitor cell populations contribute to tissue repair irrespective of the population of origin (Page et al., 2013). Common to all the stem cell populations identified so far in the epidermis is the absence of Sca-1 expression, which is interestingly as Sca-1 expression marks stem/progenitor in the hematopoietic system (Bradfute et al., 2005; Ito et al., 2003), muscle (Asakura et al., 2002; Jankowski et al., 2001), heart (cardiac stem cells) (Oh et al., 2003; Wang et al., 2014), mammary gland (Welm et al., 2002), liver (Wright et al., 2008) and prostate (Xin et al., 2005). Sca-1 belongs to the Ly6 gene family encoding highly homologous, glycosyl-phosphatidylinositol-anchored membrane proteins (van de Rijn et al., 1989). Although the precise function of Sca-1 remains unclear, Sca-1 has in several studies been shown to play a role in proliferation and differentiation (Epting et al., 2008a; Epting et al., 2004; Henderson et al., 2002; Jensen et al., 2008; Mitchell et al., 2005).

Abbreviations: Sca-1, Stem Cell Antigen-1; DMBA, 7,12-Dimethylbenz[*a*]anthracene; TPA, 2-*O*-tetradecanoylphorbol-13-acetate; EdU, 5-Ethynyl-2-deoxyuridine; TGF-β, transforming growth factor-β.

* Corresponding author.
E-mail addresses: rikkchis@rm.dk (R. Christensen), do2112@cumc.columbia.edu (D.M. Owens), acf@mbg.au.dk (A.C. Füchtbauer), gunnarssonanders@live.se (A. Gunnarsson), metramsi@rm.dk (M. Ramsing), emf@mbg.au.dk (E.-M. Füchtbauer), uffejens@rm.dk (U.B. Jensen).

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Sca-1 is highly expressed in the interfollicular epidermis outside the stem cell niches where most proliferating keratinocytes reside (Jensen et al., 2008; Triel et al., 2004) and expression has also been detected in other non stem cell populations such as proliferating myoblasts (Mitchell et al., 2005) and mature T cells (Spangrude et al., 1988). Several malignant tissues show elevated levels of Sca-1 expression including retinoblastomas (Seigel et al., 2007), prostate tumours (Xin et al., 2005), mammary tumours (Grange et al., 2008; Li et al., 2003; Yin et al., 2009), and chronic myeloid leukemia (Perez-Caro et al., 2009).

It has furthermore been speculated whether Sca-1 plays a role in stem cell self-renewal. Thus, some experiments with Sca-1 −/− knock-out mice investigating hematopoietic and mesenchymal progenitor cells have pointed to a role for Sca-1 in self-renewal (Bonyadi et al., 2003; Ito et al., 2003). However, other studies on hematopoietic stem cells and muscle stem cells indicate that Sca-1 does not seem to affect self-renewal (Bradfute et al., 2005; Kafadar et al., 2009). A complex phenotype has been observed in Sca-1 −/− knock-out mice including defects in the regulation of lineage commitment in hemapoietic stem cells (Bradfute et al., 2005), osteoporosis, reduced muscle size in aging KO mice (Bonyadi et al., 2003) and delayed muscle repair after injury (Epting et al., 2008b). In mammary tumor cells, Sca-1 has been shown to regulate cell migration, cell adhesion to several extracellular matrix substrates and tumor development in early lesions (Batts et al., 2011) and a study using a mammary adenocarcinoma cell line could demonstrate that Sca-1 suppresses GDF10-dependent TGF-β signaling by disrupting the heterodimerization of the TGF-β receptors (Upadhyay et al., 2011). Additionally, TGF-β acts as a negative regulator of Sca-1 expression in both myoblasts and splenic T cells (Long et al., 2011).

Since lack of Sca-1 seems to be a common factor for most stem cell populations in the hair follicle, we hypothesized that overexpression of Sca-1 in stem cells would disturb normal hair follicle generation and maintenance and possibly tumour formation. Therefore, to explore the role of Sca-1 in normal and malignant skin we generated mice that overexpress Sca-1 in the hair follicle progenitor cells that are normally Sca-1 negative. Here we show that overexpression of Sca-1 in the bulge and upper isthmus stem cells decreases the incidence of benign tumours but seems to increase the frequency of progression from benign to malignant tumour. No effect was observed on hair follicle morphogenesis.

2. Materials and methods

2.1. Vector construction

To produce transgenic mice expressing Sca-1 in the basal layer of the skin we utilized a CRE-regulated Rosa26-DEST knock-in vector system (Hohenstein et al., 2008) to create an altered Rosa26 locus, *Rosa26*TM$^{116(Ly6a)Emfu}$. The targeting vector Rosa26-DEST-Sca-1 (hereafter Rosa-Sca1) was produced by a 2-step procedure. The 0.7 kb full-length mouse Sca-1 cDNA fragment was isolated from plasmid pCMV-Sport6-Sca1 (RZPD) and blunt end ligated into the pENTR11 vector (Invitrogen, Life Technologies, Naerum, DK) to obtain the vector pENTR-Sca-1. The pENTR-Sca-1 then served as entry vector and pRosa26-DEST as destination vector in a Gateway LR clonase reaction (Invitrogen) to yield the pROSA26-DEST-Sca-1 vector. The LR clonase enzyme mix was transformed into Stbl3 *E. coli* cells and grown at 30 °C. The construct was confirmed by sequencing.

2.2. ES cell culture

CJ7 embryonic stem cells, derived from 129S1/Sv mice (Swiatek and Gridley, 1993), were electroporated with 25 μg *Kpn*I-linearized targeting vector pROSA26-DEST-Sca-1 and screened for resistance towards G418 (350 μg/ml). DNA from individual clones was isolated and digested with *Eco*RV according to (Ramirez-Solis et al., 1993) and subsequently analyzed by Southern blot. Targeting efficiency was 11%.

To test the expression level of Sca1 from the ROAS26 promoter, the neomycin phosphotransferase expression casette was excised in five selected clones by transient expression of Cre recombinase by electroporation.

Two days after transfection, the pools of Cre transfected cells were harvested by trypsinisation and plated on gelatinized plates for 45 min to remove feeder cells. The non-attached ES cells were stained with PE-conjugated Ly-6A/E (Sca-1, BD A/S, Albertslund, DK) and subjected to flow cytometry analysis.

2.3. Transgenic mice

10–12 ES cells from one positive clone were injected into B6D2F2 blastocysts (Wertz and Füchtbauer, 1994), which were then transfered to pseudopregnant NMRI female mice that gave birth to chimeric animals. Male chimeras were mated with C57Bl/6J female mice and agouti offspring (indicating germ line transmission of the manipulated 129S1/Sv ES cells) were tested for the presence of the Rosa-Sca1 mutation by PCR using genomic tail DNA.

Heterozygous offspring from the chimeras was backcrossed ten generations with C57Bl/6j, designated *Rosa*^{Sca1}. The resulting mice were crossed with K14-Cre mice (Tg(KRT14-cre)1Amc/J, The Jackson Laboratory, Bar Harbor, Main, USA) to obtain mice heterozygous for both the K14-Cre and Rosa-Sca1 alleles, designated *Rosa*^{Sca1}*:Cre*$^{+}$. These mice were then further crossed with a heterozygous *Rosa*^{Sca1} mouse to obtain mice homozygous for the Rosa-Sca1 allele and with and without one copy of the K14-Cre construct, designated *Rosa*$^{Sca1/Sca1}$*:Cre*$^{+}$ and designated *Rosa*$^{Sca1/Sca1}$*:Cre*$^{-}$ respectively. For the experiments, siblings homozygous for Rosa-Sca1 and heterozygous for K14-Cre (*Rosa*$^{Sca1/Sca1}$*:Cre*$^{+}$) were crossed with siblings homozygous for the Rosa-Sca1 and without Cre (*Rosa*$^{Sca1/Sca1}$*:Cre*$^{-}$) in order to obtain a 50/50 ratio of *Rosa*$^{Sca1/Sca1}$*:Cre*$^{+}$ and *Rosa*$^{Sca1/Sca1}$*:Cre*$^{-}$ mice.

Mice were housed under a light/dark cycle of 12 h with free access to food and water, and bred under specific pathogen-free conditions. All animal experiments were conducted in accordance with institutional guidelines and approved by the Animal Experiments Inspectorate in Denmark.

2.4. Genotyping

Presence of the Rosa-Sca1 and K14-Cre transgenes were determined in a multiplex PCR on DNA isolated from a tail biopsy by using the REDExtract-N-Amp Tissue PCR kit (Sigma-Aldrich, St. Louis, MO, USA). The identification of the Rosa-Sca1 transgene was performed by using the primers Rosa forward and Sca1 seq reverse flanking the upper cloning site in the transgenic construct and the primers IMR1084 and IMR 1085 were used in order to verify the integration of the K14-Cre transgene. Actin and IMR 42/43 primers were used as a control of the DNA preparation. The sequences of the primers are: Rosa forward: 5′-CATCAAGCTGATCCGGAACCC-3′; Sca1-seq reverse: 5′-CTGCACACA GTAGGGCCACAAG-3′; actin sense: 5′-CTGTGCTGTCCCTGTATGCC-3′; actin antisense: 5′-GTGGTGGTGAAGCTGTAGCC-3'; IMR1084: 5′-GCGGTCTGGCAGTAAAAACTATC-3′; IMR1085: 5′-GTGAAACAGCA TTGCTGTCACTT-3′; IMR42: 5′-CTAGGCCACAGAATTGAAAGATCT-3′; IMR43: 5′-GTAGGTGGAAATTCTAGCATCATCC-3′. The DNA was amplified by using the REDExtract-N-Amp Tissue PCR kit according to the manufacturer's instructions. Amplification parameters were as follows – after an initial denaturation step at 94 °C for 3 min, amplification was performed for 35 cycles at 94 °C for 30 s, 59 °C for 30 s and 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min.

2.5. Flow cytometry

Primary adult epidermal keratinocytes were isolated from 7 week old female mice as previously described (Nowak and Fuchs, 2009). Freshly isolated epidermal keratinocytes were suspended in 0.1% BSA/