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Abnormal differentiation, hyperplasia and embryonic/perinatal lethality in BK5-T/t transgenic mice

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Abstract

The cell-of-origin has a great impact on the types of tumors that develop and the stem/progenitor cells have long been considered main targets of malignant transformation. The SV40 large T and small t antigens (T/t), have been targeted to multiple differentiated cellular compartments in transgenic mice. In most of these studies, transgenic animals develop tumors without apparent defects in animal development. In this study, we used the bovine keratin 5 (BK5) promoter to target the T/t antigens to stem/progenitor cell-containing cytokeratin 5 (CK5) cellular compartment. A transgene construct, BK5-T/t, was made and microinjected into the male pronucleus of FVB/N mouse oocytes. After implanting ~1700 embryos, only 7 transgenics were obtained, including 4 embryos (E9.5, E13, E15, and E20) and 3 postnatal animals, which died at P1, P2, and P18, respectively. Immunohistological analysis revealed aberrant differentiation and prominent hyperplasia in several transgenic CK5 tissues, especially the upper digestive organs (tongue, oral mucosa, esophagus, and forestomach) and epidermis, the latter of which also showed focal dysplasia. Altogether, these results indicate that constitutive expression of the T/t antigens in CK5 cellular compartment results in abnormal epithelial differentiation and leads to embryonic/perinatal animal lethality.

Keywords

Cytokeratin 5; stem cells; differentiation; SV40 large T; tongue papillae; transgenic

Introduction

The cell-of-origin has a great impact on the types of tumors that develop (Perez-Losada and Balmain, 2003) and the stem/progenitor cells have long been considered main targets of

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malignant transformation (Reya et al., 2001). For example, oncogenic Ras targeted to the cytokeratin 10 (CK10) (Bailleul et al., 1990) or CK1 (Greenhalgh et al., 1993), differentiated suprabasal epidermis in transgenic mice leads to mostly benign papillomas that depend on wounding as a critical secondary event and rarely progress to malignancy. In contrast, the same mutant Ras targeted to the stem/progenitor cell-containing CK5 compartment results in wounding-independent keratoacanthomas that have a high frequency of spontaneous progression to squamous cell carcinomas (Brown et al., 1998). Similarly, the same combination of transforming genes (the SV40 T/t, H-ras, and hTERT) targeted to two different populations of human mammary epithelial cells results in tumors of distinct histological types (Ince et al., 2007). These observations help explain the inter-patient tumor heterogeneity. Tumor heterogeneity is also reflected as intra-tumor cellular heterogeneity in that most tumors comprise a variety of cell types of divergent phenotypic and functional properties. Emerging evidence suggests that the cellular heterogeneity in tumors may be created by a small population of stem-like cells called cancer stem cells (CSCs). Putative CSCs have been reported in several human cancers (Al-Hajj et al., 2003; Bonnet and Dick, 1997; Patrawala et al., 2005; Prince et al., 2007).

The SV40 T and t antigens are among the most potent transforming genes and have been widely used in transgenic models to study tumor development (Jensen et al., 1993; Maroulakou et al., 1994; Greenberg et al., 1995; Magdaleno et al., 1997; Connolly et al., 2003; Deeb et al., 2007). The T antigen abrogates the functions of p53 and pRb (Ahuja et al., 2005) whereas the t protein deregulates protein phosphatase 2A (Arroyo and Hahn, 2005), which together leads to efficient cellular transformation. In most of the transgenic studies using either the SV40 early region, which encodes both T and t, or the T alone, the transgene(s) were targeted to the differentiated cellular compartments and tumors were observed without apparent defects in animal development. For example, the rat prostatic steroid binding protein [C3(1)] gene promoter driving T/t expression resulted in prostate and breast hyperplasia and adenocarcinomas (Maroulakou et al., 1994). Similarly, targeting T/t to the differentiated probasin-expressing prostate luminal cells resulted in aggressive prostate adenocarcinomas, i.e., TRAMP (Greenberg et al., 1995). Finally, transgenic expression of T in Clara cells led to lung tumor development¹⁶ and of SV40 early region in ovarian epithelial cells resulted in bilateral ovarian tumors (Connolly et al., 2003). These transgenic studies provide convincing evidence that differentiated mouse cells *can* be transformed by powerful oncogene(s) such as T or T/t. Recently, a T/t gene signature was uncovered by microarray analysis, which is activated in tumors with aberrant p53 or Rb expression and is highly predictive of the aggressiveness of human breast, lung, and prostate tumors (Deeb et al., 2007).

We wonder how targeting the SV40 T/t antigens to a stem/progenitor cell-enriched cellular compartment might impact animal and tumor development. To this end, we chose the CK5 cellular compartment. CKs are the intermediate filament proteins expressed primarily in epithelial cells (Gu and Coulombe, 2007). There are two types of CKs, acidic type I (CK9–0) and basic/neutral type II (CK1–8), and one member of type II usually pairs with a member of the type I CKs to form functional keratin filaments (Gu and Coulombe, 2007). CK5 and CK14 are the major keratins that form heteromeric complexes in the basal epidermis (Byrne and Fuchs, 1993; Tumber et al., 2004) as well as in the basal layer of some internal epithelial organs such as breast and prostate (Bocker et al., 2002; Sawicki and Rothman, 2002). Germline mutations in either *CK5* or *CK14* genes cause human diseases like epidermolysis bullosa simplex (EBS; a blistering skin disorder involving cytolysis in the basal layer of epidermis) (Schuilenga-Hut et al., 2003) suggesting overlapping functions of CK5 and CK14. On the other hand, although both Krt5 (K5) and Krt14 (K14) deficient mice suffer from EBS, only *K5*^{−/−} mice are neonatally lethal (Peters et al., 2001), suggesting that CK5 might play a more prominent role than CK14 in regulating epithelial tissues. Alternatively, some other type I keratins may substitute CK14. Indeed, in the absence of CK14, CK15 can establish a functional

keratin network with CK5 in suprabasal keratinocytes in the mouse epidermis (Lloyd et al., 1995).

CK5 and CK14 are expressed in the basal epidermis and bulge region of the hair follicles where stem/progenitor cells localize (Byrne and Fuchs, 1993; Tumber et al., 2004). In mammary and prostatic glands there also exists a CK5/CK14-positive cell layer that is thought to host tissue stem/progenitor cells (Bocker et al., 2002; Sawicki and Rothman, 2002). This expression pattern suggests that although the majority of the CK5/CK14-expressing cells probably represent differentiated basal cells, a small subset may mark stem/progenitor cells that possess self-renewing and high proliferative capacities. In support of this role, the CK5-expressing, slow-cycling cells purified from the bulge region in transgenic mice are found to possess both gene expression profiles and functional characteristics of stem cells (Tumber et al., 2004). Furthermore, the CK5⁺ cells from the breast and prostate are clonogenic and appear to be able to generate different cell lineages in *in vitro* assays (Bocker et al., 2002; Sawicki and Rothman, 2002). Finally, putative CSCs in prostate adenocarcinomas (Patrawala et al., 2006) and head and neck squamous cell carcinomas (Prince et al., 2007) are also CK5-positive.

Here we show that transgenic expression of the SV40 T/t in the CK5 cellular compartment results in abnormal cellular differentiation and severe hyperplasia in some CK5 tissues and causes embryonic/perinatal lethality.

Materials and Methods

Establishment and characterization of the BK5-T/t transgene construct

A plasmid containing the entire SV40 genome (pSV2X72; courtesy of Dr. Janet Butel, Baylor College of Medicine, Houston, TX), was used to obtain the coding region of SV40 T/t via PCR amplification using Tag forward primer (5'-GCTATTCCAGAAGTAGTGAGGAGGC-3') and Tag reverse primer (5'-AAGCTGCAAT AAACAAGTTAACAACAAC-3'), as illustrated in Supplementary Figure S1A. The PCR primers contain restriction sites Not I and Nhe I, respectively, and PCR was performed as follows: 95°C for 3 min followed by 30 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 2.5 min, giving a ~2.6-kb product. The Not I/Nhe I PCR fragment was subcloned into the pBK5.197 vector (Klug et al., 2000; Matsumoto et al., 2003) in which the bovine keratin 5 (BK5) promoter (Ramírez et al., 1994) drives transgene expression to the CK5 compartment. The resultant BK5-T/t construct (Supplementary Figure S1A) was characterized by restriction digestion and sequencing.

To functionally characterize the transgene, the CK5-expressing NHP progenitor cells (Bhatia et al., 2005) were transfected, using Fugene 6, with the purified, 9.5 kb Kpn BK5-T/t fragment (Supplementary Figure S1A). Seventy-two h later, cells were pulsed with 5 μ M BrdU (4 h), fixed and then processed for BrdU and T immunostaining. The basic staining protocols were previously described (Bhatia et al., 2008).

Pronuclear injection and genotyping

Donor FVB/N female mice (Harlan Sprague Dawley, Indianapolis, IN) were injected (i.p.) with 5U pregnant mare's serum (PMS; Sigma, #G4877), and 46–48 h later, were given (i.p.) with 2.5–5U human chorionic gonadotropin (HCG; Sigma, #CG-10). After HCG injection, the females were mated with fertile FVB/N males overnight and, next morning, the plugged FVB/N females were sacrificed and one-cell embryos were collected. Purified Kpn fragment (Supplementary Figure S1A) was microinjected into the male pronuclei and surviving embryos were transferred to pseudopregnant ICR females, which were generated by mating ICR female mice (Harlan Sprague Dawley) with vasectomized B6D2 F1 males (National Cancer Institute, Bethesda, MD).

Embryos and postnatal animals were genotyped by PCR using primers that amplify a ~400-bp fragment of the rabbit β -globin intron (see Supplementary Figure S1 A for illustration). The primers were 5'-GTGTTGTTTAGAATGGGAAGATGT-3' (forward) and 5'-TAAAGAGAAAGGCAGGA TGATGA -3' (reverse). The PCR of mouse β -casein was used as the internal control using the primers 5'-GATGTGCTCCAGGCTAAGTT-3' (forward) and 5'-AGAAACGGATGTTGTGGAGT-3' (reverse).

Histological and immunohistological (IHC) analyses

The transgenic and wild-type (WT) embryos and tissues harvested from the postnatal transgenic and WT animals were fixed in formalin for 48 h followed by 70% ethanol and embedding in paraffin. Sections (4 μ m) were cut and stained with hematoxylin and eosin (HE). For IHC, sections were deparaffinized and hydrated and endogenous peroxidase activity was blocked with 3% H₂O₂ in water for 10 min. Antigen retrieval was performed with 10 mM citrate buffer (pH 6.0) for 10 min in a microwave oven followed by a 20-min cool down and thorough wash. Slides were incubated with Biocare Blocking Reagent (#BS966M with casein in the buffer) for 10 min to block non-specific binding. Slides were incubated with various primary antibodies including: SV40 Tag (1:100; EMD Biosciences, #DP02), CK5 (1:500; Covance, #PRB-160P), CK10 (1:500; Covance, #PRB-159P), CK13 (1:200; AbCam, #ab16112), filaggrin (1:1000; BabCo, #PRB-417p), involucrin (1:1000; BabCo, #PRB-140c), loricrin (1:1000; BabCo, #PRB-145p), monoclonal anti-SV40 t (1:1000; PAb280, Oncogene Sciences), caspase-3 (1:2000; R&D, #AF835), and Ki67 (1:200; DAKO, #M7249), for 30 min at room temperature. Slides were washed in phosphate buffer twice and then incubated in biotinylated goat-anti-rabbit or mouse IgG (Vector Laboratories, Burlingame, CA) at a 1:500 dilution for 30 min at room temperature. After thorough washing, slides were incubated with SA-HRP (BioGenex, San Ramon, CA) for 30 min at room temperature followed by washing. Finally, slides were incubated with BioGenex DAB substrate and color development was closely monitored under a microscope. Slides were lightly counterstained with hematoxylin.

Measurement of epithelial thickness and proliferative cells and statistical analysis

The approximate thickness of epidermis, tongue epithelium, and palate mucosa was measured on HE-stained sections using a stage micrometer (1 $\times$ 0.01 mm, Fisher) under a light microscope (LEICA DMLS) at a magnification of 400 $\times$. On average, 7–10 fields and 25–50 independent measurements per section and a total of 4–6 sections were analyzed for each tissue. The Ki67-positive, proliferating cells were counted in a total of 2,000–4,000 basal cells from 6 individual sections for each tissue. The Student's *t*-test was performed for thickness measurements and the chi-squared test was used for Ki67-positive cells.

Results and discussion

CK5 is expressed in epidermis and many internal organs in wild-type FVB/N mice

CK5 is well known to be expressed in epidermis, skin appendages, and some epithelial tissues (Byrne and Fuchs 1993). To facilitate analysis of transgene expression in our animals, we first carried out a survey of CK5 expression in ~3 month-old FVB/N mice. This IHC analysis revealed CK5 expression in epidermis and hair follicles as well as in multiple epithelial tissues including: part of the digestive system (salivary gland, oral mucosa, tongue, esophagus, forestomach, and pancreas), upper respiratory tract (e.g., nasal epithelium and bronchus), urinary/reproductive tract (bladder, vagina, and cervix), secondary sex organs (breast and prostate), and cornea (fig. S1; table S1). Among the non-epithelial tissues examined, only thymus prominently expressed CK5, consistent with earlier studies (Klug et al., 2000).

Constitutive expression of the SV40 T in the CK5 cellular compartment results in embryonic / perinatal lethality

To explore the potential involvement of the CK5 cellular compartment in tumorigenesis, we made the BK5-T/t transgene construct (Fig. 1A). The BK5 promoter has been widely used to drive transgene expression specifically in CK5-expressing cells (Ramírez et al., 1994). We first characterized the functionality of the transgene construct by transfecting the ~9.5-kb BK5-T/t Kpn fragment (Fig. 1A) into the CK5-expressing NHP6 (normal human prostate epithelial cells; strain 6) progenitor cells (Bhatia et al., 2005) and stained the cells with a T-specific monoclonal antibody (mAb; clone PAb416), which was raised against the Ile83 – Lys128 peptide of the T protein and does not cross-react with the t antigen (the company product sheet). This mAb specifically detected the 94-kD T protein in 293T cells in our previous studies (Bhatia et al., 2008). Since there are currently no high-quality commercial t-specific antibodies, we mainly used the PAb416 mAb, in this study, to characterize the T protein expression, as in all previous T/t transgenic studies (Maroulakou et al., 1994; Greenberg et al., 1995; Magdaleno et al., 1997; Connolly et al., 2003; Deeb et al., 2007). In the transfected NHP6 cells, we observed T protein expression mostly in the nuclei (Fig. 1B, arrowhead) although in some cells it was also detected throughout the cells (Fig. 1B, arrow; Fig. 1C, asterisk). Importantly, expression of the T protein promoted NHP6 cell proliferation as evidenced by the significantly increased numbers of BrdU-positive cells in T-expressing cells (Fig. 1C and 1D). These observations are consistent with the T/t proteins being potent oncogenes (Ahuja et al., 2005; Arroyo and Hahn, 2005) and suggest that our BK5-T/t transgene construct is biologically functional.

The 9.5-kb Kpn transgene fragment was subsequently microinjected into the male pronucleus of FVB/N embryos. Of the ~3,000 eggs injected (over a span of ~15 months), 1,749 survived and were transferred to a total of 49 pseudopregnant recipient females (table S2). Strikingly, only 7 transgenics, including 4 embryos (E9.5, E13, E15, and E20) and 3 postnatal (P1, P2, and P18) animals were identified. This represented 0.4% positive transgenesis, which was significantly lower than the ~3% average that our Transgenic Facility Core had obtained in the past several years with the other 12 transgenic constructs. The low percentage of the transgenics suggests that the T/t expression in CK5 cellular compartment is embryonically/perinatally lethal. Indeed, some of the absorbed embryos recovered were positive for the transgene (not shown) and all three transgenic animals that were born live subsequently died at P1, P2, and P18, respectively (table S2; see below). These results are consistent with the observations that the *K5* gene is turned on early during development (i.e., around E9.25 - E9.5) and that most *K5*^{-/-} mice die perinatally (Byrne et al., 1994; Peters et al., 2001). These results (Byrne et al., 1994; Peters et al., 2001; this study) imply that the CK5 cellular compartment is important in animal development.

T protein is expressed in epidermis and other CK5+ tissues in transgenic embryos/animals

Subsequently, we characterized the transgene (i.e., T) expression and compared with the endogenous CK5 expression. The results revealed T protein expression in the CK5-positive tissues/organs in all 7 transgenic embryos and animals (Table 1; Fig. 2; fig. S2). In E9.5 transgenic embryo, the T protein was detected in surface ectoderm (Fig. 2A, b and c; which gives rise to future epidermis along with glands, hair, and nail; epithelium of the mouth and nasal cavity, salivary glands, and glands of mouth and nasal cavity; enamel organ; epithelium of pineal and pituitary glands; and lens and cornea of the eye), first branchial arch (Fig. 2A, e and f; which develops into jaws and palate, among others), olfactory placode (Fig. 2A, h and i; which develops into olfactory epithelium of the nose), and limb bud epithelium (Fig. 2A, k-l), all of which expressed endogenous CK5 (Fig. 2A, a, d, g, and j). In E15 transgenic embryo, T protein was observed in surface ectoderm, forelimb, primordia of follicles of vibrissae, corneal epithelium, olfactory epithelium, tongue epithelium, esophagus, stomach, and thymus, all of which also expressed endogenous CK5 (Table 1; Fig. 2B; data not shown). Similar

concordant expression of T and endogenous CK5 was also observed in all other transgenic embryos and animals (Table 1; Fig. 2C; fig. S2). The T protein was also detected in CK5-positive mesothelial cells lining some internal organs such as the heart and liver (not shown). In all these cases, the T protein was detected nearly exclusively in the cell nuclei (Fig. 1, Supplementary Figure S3).

Interestingly, in E9.5 – E20 transgenic embryos and P1 transgenic animal, we consistently observed the T protein expression in several brain regions negative for CK5 staining (Table 1; fig. S3). In P1 animal, we also observed T-positive cells in CK5-negative lung parenchyma (fig. S3, p-r). Most of the T-positive/CK5-negative cells presented mesenchymal cell morphology and were detected mainly in cranial mesenchyme (fig. S3, f, i, and l) although in E9.5 embryo and the P1 animal such cells were detected as neuroepithelial-like cells lining the ventricles (fig. S3, c and o; Table 1). It is presently unclear how these ‘ectopic’ transgene expressions were brought about. Since these cells were mostly observed in the developing brain, which has extensive neural stem cell migration and epithelial-mesenchymal transition or EMT (Kang and Svoboda, 2005), these T-positive/CK5-negative cells might be cells that were in transition. Presumably, the T protein might have a longer half-life than the endogenous CK5 such that epithelial cells undergoing EMT have lost CK5 expression but the T protein expression has persisted longer.

In all IHC analyses, the T-positive cells were not associated with increased apoptosis as revealed by active caspase-3 staining (data not shown).

Aberrant differentiation and hyperplasia in upper digestive tissues including tongue papillae, oral mucosa, esophagus, and forestomach in the transgenic embryos/animals

We compared the transgenic and WT embryos/neonates in attempt to gain some insight on the transgene expression-induced embryonic/perinatal lethality. To our surprise, the 4 transgenic embryos were grossly normal compared to the age-matched WT embryos (fig. S4; data not shown). Nevertheless, the three postnatal transgenics had some noteworthy phenotypes. For example, all of them lacked milk in their stomach during necropsy. The P1 transgenic animal showed severe gasping prior to its death. Interestingly, this animal had ‘ectopic’ transgene expression in the lung and its pulmonary alveoli seemed to be under-developed compared to the littermate WT control (fig. S3q-r; data not shown). Finally, the P18 transgenic animal was much smaller (3.97 g) compared to the WT littermates (averaged 6.94 g).

Histological and IHC analyses revealed developmental defects in transgenic tongue, oral (palate) mucosa, esophagus, and forestomach (Fig. 3, Fig. 4; fig. S5; data not shown) that might help explain the lack of milk and smaller body sizes in transgenics. In WT E20 embryo to P18 animals, an age-related maturation in filiform papillae was observed in the tongue (Fig. 3A, b,f,j,n). In sharp contrast, E20 to P18 transgenic tongues completely lacked the filiform papillae development and, instead, showed prominent epithelial hyperplasia (Fig. 3A, d,h,l,p). Quantification revealed thickened tongue epithelia in the transgenics (Fig. 3C, a and b). For example, the E20 transgenic and WT tongue epithelial thickness was $74.4 \pm 1.1 \mu\text{m}$ vs. $53.5 \pm 1.1 \mu\text{m}$ (mean $\pm$ SEM; $P < 0.0001$). The P1 transgenic and WT tongue epithelial thickness was $66.5 \pm 2.5 \mu\text{m}$ vs. $46.7 \pm 1.1 \mu\text{m}$ ($P = 0.007$). Similarly, the P18 transgenic and WT tongue epithelial thickness was $82.3 \pm 2 \mu\text{m}$ vs. $69.5 \pm 2 \mu\text{m}$ ($P = 0.0009$). The hyperplasia in the tongue epithelia was associated with significantly increased cell proliferation (i.e., Ki67+) in the basal layer (Fig. 3Cc). Microscopically, the Ki67-positive cells were localized nearly exclusively in the basal layer in WT tongue epithelium; however, in the transgenic tongue epithelia, the Ki67-positive cells were detected across all cell layers (data not shown; see Fig. 3Dd for an example of Ki67 staining in the palate).

Abnormal tongue papilla differentiation and epithelial hyperplasia were confirmed by immunohistochemical staining for CK13, a CK normally expressed in the differentiating and differentiated lingual epithelium (Iwasaki et al., 2006). As shown in Fig. 4Ac, the P1 WT tongue epithelium showed well-spaced developing filiform papillae covered at the outmost by a CK13-positive, acellular, and cornified keratin layer. In contrast, the P1 transgenic lingual epithelium lacked filiform papillae and had reduced outmost acellular layer; instead, the CK13-positive cellular layer was significantly expanded (Fig. 4Ad). These alterations became more pronounced in the P18 transgenic tongue epithelium, which was devoid of the differentiated, CK13-positive filiform papillae accompanied by prominent hyperplasia in the CK13-positive cellular compartment (Fig. 4Bd). These observations suggest that the T/t expression not only promotes cell proliferation, as supported by increased Ki67-positive cells (Fig. 3Cc), but also disrupts the normal differentiation in lingual epithelium. In further support of this latter suggestion, we observed that in the WT tongue, both the CK5 (Figs. 4Ae and 4Be) and Ki67 (not shown) cells were, as expected, mostly restricted to the basal layer. In sharp contrast, in the transgenics, both Ki67 (not shown) and CK5 (Figs. 4Af and 4Bf) positive cells, much like the T-expressing cells, were observed across the whole tongue epithelium, indicating a loss of the normal, polarized and stratified epithelial organization. Therefore, in the tongue epithelium, the transgene expression results in both defective differentiation (leading to lack of filiform papillae) and epithelial hyperplasia (resulting from both increased cell proliferation and abnormal differentiation). Interestingly, the fungiform papillae (Fig. 4C, a-d) and the taste buds embedded within, which were CK13-negative (Fig. 4C, e-h), also displayed abnormal differentiation in the transgenic tongues (compare Fig. 4C, d and h vs. c and g).

Epithelial hyperplasia and aberrant differentiation were also observed in E20 to P18 transgenic palate mucosa (Figs. 3B, 3Da-b, 4Ag-j, and 4Bg-j; data not shown). For example, the E20 transgenic and WT palate epithelial thickness was $64.1 \pm 3.1 \mu\text{m}$ vs. $48.9 \pm 2.0 \mu\text{m}$ (mean $\pm$ SEM; $P = 0.003$). The P2 transgenic and WT palate epithelial thickness was $74.6 \pm 2.1 \mu\text{m}$ vs. $54.3 \pm 3.2 \mu\text{m}$ ($P = 0.003$). Again, the hyperplasia in the BK5-T/t transgenic oral mucosa was associated with increased Ki67+ cells (Fig. 3Dc), which were not only significantly increased in the basal layer but also were observed throughout the suprabasal layer that normally lacked proliferating cells (Fig. 3Dd). CK13 IHC staining revealed that the transgenic mucosa lacked the outmost CK13-positive, terminally differentiated, acellular keratin layer but instead showed a prominent expansion in the CK13-positive, differentiating cellular compartment (Figs. 4A and B; compare h and g). These results again indicate that the transgene expression disrupted the terminal differentiation in the BK5-T/t palate mucosa, as further supported by the detection of CK5-positive cells throughout the palate epithelium (Figs. 4A and B, compare j and i). Similar histological and IHC analyses also revealed abnormal differentiation and hyperplasia in esophagus (not shown) and the forestomach (fig. S5). In the transgenic forestomach, focal hyperplasia was accompanied by prominent hyperkeratosis (increased cornified keratin) in some other areas (fig. S5). In the WT forestomach, the CK5-positive cells were localized mainly in the basal layer (fig. S5, b, e, h). In contrast, the CK5-positive cells in the transgenic forestomach were often found throughout the entire hyperplastic epithelium (although the overall CK5 staining intensity was reduced) (fig. S5, b,e,h,k), indicating the loss of normally polarized and stratified epithelial organization in this tissue.

The above observations (Fig. 3, Fig. 4, fig. S5) together suggest that aberrant differentiation and hyperplasia in the upper digestive tissues (including palate mucosa, tongue papillae and taste buds, esophagus, and forestomach) presumably lead to markedly reduced food intake in perinatal transgenic animals, hence the empty stomach, smaller body, and eventual animal death. The pronounced hyperkeratosis in the transgenic forestomach likely is due to reduced food intake (Til et al., 1988).

Abnormal differentiation and hyperplasia in transgenic epidermis

As mentioned earlier, the CK5 and CK14 pair represents the major intermediate filament proteins in the epidermal basal cell layer (stratum basale) that has the regenerative potential. In BK5-T/t epidermis, the T protein was detected in the interfollicular keratinocytes (mainly basal cells but also some suprabasal cells), the outer root sheath cells of hair follicles, and the epithelial lining of sebaceous glands (Fig. 2B-C; fig. S2; data not shown). The hair follicle development and numbers and sebaceous glands in the transgenics were not significantly different from those in the WT controls (Figs. 5A and B; data not shown), consistent with a lack of apparent hair phenotype in the three postnatal transgenic animals. Nevertheless, we observed (interfollicular) epidermal hyperplasia and focal dysplasia in the transgenic mice (Fig. 5 and data not shown). As early as E20, the BK5-T/t epidermis was significantly thicker than the E20 WT epidermis ($60.6 \pm 1.0 \mu\text{m}$ vs. $51.5 \pm 1.0 \mu\text{m}$; $P = 0.025$). At P1 when the normal epidermal thinning occurs (Neufang et al., 2001), the transgenic and WT epidermal thickness was $27.8 \pm 1.0 \mu\text{m}$ vs. $16.8 \pm 1.0 \mu\text{m}$ (mean $\pm$ SEM; $P < 0.0001$; Figs. 5A and 5C). Similarly, the P18 BK5-T/t transgenic epidermis ($19.9 \pm 1.0 \mu\text{m}$; mean $\pm$ SEM) was about twice as thick as the age-matched WT epidermis ($9.8 \pm 1.0 \mu\text{m}$; $P < 0.0001$) (Figs. 5B and 5D). These results suggest that SV40 T/t expression in the CK5+ epidermis causes hyperplasia. In fact, in the P18 transgenic epidermis, focal dysplasia was often observed (e.g., Fig. 5Bd). The transgenic epidermis also showed some abnormalities in differentiation. For example, staining for CK10, an immediate early marker of epidermal differentiation (Neufang et al., 2001) revealed expanded CK10-positive cellular layer in both P1 (Fig. 5A) and P18 (Fig. 5B) BK5-T/t transgenics. Furthermore, the transgenic epidermis, especially in the P18 animal, showed hyperkeratosis with thick, CK10-positive keratin bundles deposited outside of the epidermal layer (Fig. 5Bh). The epidermal hyperkeratosis most likely developed as a consequence of abnormal differentiation in the transgenic epidermis (Neufang et al., 2001). In support, IHC analysis of expression in involucrin, loricrin, and filaggrin, which mark early to late epidermal differentiation (Neufang et al., 2001; Robitaille et al., 2005), revealed abnormal differentiation patterns in the P1 transgenic epidermis (fig. S6).

In several other CK5+ tissues examined including ureter, bladder, pancreas, thymus, trachea, and bronchus, we observed mild hyperplasia in the transgenic trachea and bronchus (data not shown).

In summary, the observations that oncogene(s) targeted to different cellular compartments generate different types of tumors and the long-held view that stem/progenitor cells may represent the main targets of malignant transformation prompted us to start the current project by directing the SV40 T/t antigens to the stem/progenitor cell-containing CK5 compartment. Our initial expectation was that the BK5-T/t transgenic animals, in a short period of time, might develop aggressive malignant tumors in the skin and some other CK5+ tissues such as breast and prostate, which would allow us to compare with the tumors derived from targeted T/t expression in more differentiated cellular compartments (see introduction). Somewhat unexpectedly, however, the BK5-T/t transgenic animals do not survive beyond the early postnatal period. Since the only three transgenic mice that were born alive all died within 3 weeks after birth, it is possible that most transgenics died embryonically. This possibility would be consistent with the activation of *K5* gene expression around E9.25 - E9.5 in mice and was supported by the recovery of transgene-positive partially absorbed embryos. The embryonic/perinatal lethality in the BK5-T/t transgenics is also consistent with pRb being important for murine development (Clarke et al., 1992). Interestingly, somatic deletion of *Rb* in epidermis using K14-Cre results in epidermal hyperplasia, aberrant differentiation, and hyperkeratosis (Ruiz et al., 2004).

The exact reason underlying the embryonic/perinatal lethality in BK5-T/t transgenics remains unclear. Although the death of postnatal transgenics might be related to severely reduced food

intake caused by abnormal development and severe hyperplasia in the upper digestive system (mainly tongue papillae and taste bud, palate mucosa, esophagus, and forestomach) and defective skin differentiation, these developmental problems are unlikely to account for the embryonic lethality. Since the T or T/t antigens targeted to multiple differentiated cellular compartments in transgenic mice, although leading to tumor development, do not generally result in significant developmental defects and animal lethality (Maroulakou et al., 1994; Greenberg et al., 1995; Magdaleno et al., 1997; Connolly et al., 2003; Deeb et al., 2007), our results implicate an important role of the CK5 cell compartment, which contains stem/progenitor cells that can differentiate into functional cell types (such as keratinocytes in the suprabasal epidermis and secretory cells in the breast and prostate), in early animal development. This implication is consistent with the perinatal lethality observed in the K5 knockout mice (Peters et al., 2001). The pronounced hyperplasia in most CK5+ transgenic epithelial tissues and focal dysplasia in the epidermis also suggests that the CK5 compartment might be susceptible to tumorigenic transformation. In support of this possibility, many tumor-promoting genes, e.g. c-Src (Matsumoto et al., 2003), E2F1 (Pierce et al., 1999), cyclooxygenase 2 (Klein et al., 2005), compartment, induce hyperplasia and tumor development in transgenic animals. We are currently when overexpressed in the CK5 cellular developing a temporally controlled, inducible tissue-specific K5 transgenic model, which, if successful, would allow us to circumvent embryonic/perinatal lethality and to observe potential tumor development.

Supplementary Material

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Abbreviations

BK5, bovine keratin 5; CK5, cytokeratin 5; CSCs, cancer stem cells; SCs, stem cells; SV40 T/t, Simian Virus 40 large T and small t antigens.

References

- Ahuja D, Saenz-Robles MT, Pipas JM. SV40 large T antigen targets multiple cellular pathways to elicit cellular transformation. *Oncogene* 2005;24:7729–7745. [PubMed: 16299533]
- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 2003;100:3983–3988. [PubMed: 12629218]
- Arroyo JD, Hahn WC. Involvement of PP2A in viral and cellular transformation. *Oncogene* 2005;24:7746–7755. [PubMed: 16299534]
- Bailleul B, Surani MA, White S, Barton SC, Brown K, Blessing M, Jorcano J, Balmain A. Skin hyperkeratosis and papilloma formation in transgenic mice expressing a ras oncogene from a suprabasal keratin promoter. *Cell* 1990;62:697–708. [PubMed: 1696852]
- Bhatia B, Multani AS, Patrawala L, Chen X, Calhoun-Davis T, Zhou J, Schroeder L, Schneider-Broussard R, Shen J, Pathak S, Chang S, Tang DG. Evidence that senescent human prostate epithelial cells enhance tumorigenicity: Cell fusion as a potential mechanism and inhibition by p16INK4a and hTERT. *Int J Cancer* 2008;122:1483–1495. [PubMed: 18059027]

- Bhatia B, Tang S, Yang P, Doll A, Aumuller G, Newman RA, Tang DG. Cell-autonomous induction of functional tumor suppressor 15-lipoxygenase 2 (15-LOX2) contributes to replicative senescence of human prostate progenitor cells. *Oncogene* 2005;24:3583–3595. [PubMed: 15750631]
- Bocker W, Moll R, Poremba C, Holland R, Van Diest PJ, Dervan P, Bürger H, Wai D, Ina Diallo R, Brandt B, Herbst H, Schmidt A, Lerch MM, Buchwallow IB. Common adult stem cells in the human breast give rise to glandular and myoepithelial cell lineages: a new cell biological concept. *Lab Invest* 2002;82:737–746. [PubMed: 12065684]
- Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997;3:730–737. [PubMed: 9212098]
- Brown K, Strathdee D, Bryson S, Lambie W, Balmain A. The malignant capacity of skin tumours induced by expression of a mutant H-ras transgene depends on the cell type targeted. *Curr Biol* 1998;8:516–524. [PubMed: 9560338]
- Byrne C, Fuchs E. Probing keratinocyte and differentiation specificity of the human K5 promoter in vitro and in transgenic mice. *Mol Cell Biol* 1993;13:3176–3190. [PubMed: 7684490]
- Byrne C, Tainsky M, Fuchs E. Programming gene expression in developing epidermis. *Development* 1994;120:2369–2383. [PubMed: 7525178]
- Clarke AR, Maandag ER, van Roon M, van der Lugt NM, van der Valk M, Hooper ML, Berns A, te Riele H. Requirement for a functional Rb-1 gene in murine development. *Nature* 1992;359:328–330. [PubMed: 1406937]
- Connolly DC, Bao R, Nikitin AY, Stephens KC, Poole TW, Hua X, Harris SS, Vanderhyden BC, Hamilton TC. Female mice chimeric for expression of the simian virus 40 TAg under control of the MISIR promoter develop epithelial ovarian cancer. *Cancer Res* 2003;63:1389–1397. [PubMed: 12649204]
- Deeb KK, Michalowska AM, Yoon CY, Krummey SM, Hoenerhoff MJ, Kavanaugh C, Li MC, Demayo FJ, Linnoila I, Deng CX, Lee EY, Medina D, Shih JH, Green JE. Identification of an integrated SV40 T/t-antigen cancer signature in aggressive human breast, prostate, and lung carcinomas with poor prognosis. *Cancer Res* 2007;67:8065–8080. [PubMed: 17804718]
- Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM. Prostate cancer in a transgenic mouse. *Proc Natl Acad Sci U S A* 1995;92:3439–3443. [PubMed: 7724580]
- Greenhalgh DA, Rothnagel JA, Quintanilla MI, Orenge CC, Gagne TA, Bundman DS, Longley MA, Roop DR. Induction of epidermal hyperplasia, hyperkeratosis, and papillomas in transgenic mice by a targeted v-Ha-ras oncogene. *Mol Carcinog* 1993;7:99–110. [PubMed: 7681293]
- Gu LH, Coulombe PA. Keratin function in skin epithelia: a broadening palette with surprising shades. *Curr Opin Cell Biol* 2007;19:13–23. [PubMed: 17178453]
- Ince TA, Richardson AL, Bell GW, Saitoh M, Godar S, Karnoub AE, Iglehart JD, Weinberg RA. Transformation of different human breast epithelial cell types leads to distinct tumor phenotypes. *Cancer Cell* 2007;12:160–170. [PubMed: 17692807]
- Iwasaki S, Yoshizawa H, Aoyagi H. Immunohistochemical expression of keratins 13 and 14 in the lingual epithelium of rats during the morphogenesis of filiform papillae. *Arch Oral Biol* 2006;51:416–426. [PubMed: 16271699]
- Jensen NA, Rodriguez ML, Garvey JS, Miller CA, Hood L. Transgenic mouse model for neurocristopathy: Schwannomas and facial bone tumors. *Proc Natl Acad Sci U S A* 1993;90:3192–3196. [PubMed: 8386366]
- Kang P, Svoboda KK. Epithelial-mesenchymal transformation during craniofacial development. *J Dent Res* 2005;84:678–690. [PubMed: 16040723]
- Klein RD, Van Pelt CS, Sabichi AL, Dela Cerda J, Fischer SM, Furstenberger G, Müller-Decker K. Transitional cell hyperplasia and carcinomas in urinary bladders of transgenic mice with keratin 5 promoter-driven cyclooxygenase-2 overexpression. *Cancer Res* 2005;65:1808–1813. [PubMed: 15753378]
- Klug DB, Crouch E, Carter C, Coghlan L, Conti CJ, Richie ER. Transgenic expression of cyclin D1 in thymic epithelial precursors promotes epithelial and T cell development. *J Immunol* 2000;164:1881–1888. [PubMed: 10657637]

- Lloyd C, Yu QC, Cheng J, Turksen K, Degenstein L, Hutton E, Fuchs E. The basal keratin network of stratified squamous epithelia: defining K15 function in the absence of K14. *J Cell Biol* 1995;129:1329–1344. [PubMed: 7539810]
- Magdaleno SM, Wang G, Mireles VL, Ray MK, Finegold MJ, DeMayo FJ. Cyclin-dependent kinase inhibitor expression in pulmonary Clara cells transformed with SV40 large T antigen in transgenic mice. *Cell Growth Differ* 1997;8:145–155. [PubMed: 9040936]
- Maroulakou IG, Anver M, Garrett L, Green JE. Prostate and mammary adenocarcinoma in transgenic mice carrying a rat C3(1) simian virus 40 large tumor antigen fusion gene. *Proc Natl Acad Sci U S A* 1994;91:11236–11240. [PubMed: 7972041]
- Matsumoto T, Jiang J, Kiguchi K, Ruffino L, Carbajal S, Beltran L, Bol DK, Rosenberg MP, DiGiovanni J. Targeted expression of c-Src in epidermal basal cells leads to enhanced skin tumor promotion, malignant progression, and metastasis. *Cancer Res* 2003;63:4819–4828. [PubMed: 12941801]
- Neufang G, Furstenberger G, Heidt M, Marks F, Muller-Decker K. Abnormal differentiation of epidermis in transgenic mice constitutively expressing cyclooxygenase-2 in skin. *Proc Natl Acad Sci U S A* 2001;98:7629–7634. [PubMed: 11381142]
- Patrawala L, Calhoun T, Schneider-Broussard R, Li H, Bhatia B, Tang S, Reilly JG, Chandra D, Zhou J, Claypool K, Coghlan L, Tang DG. Highly purified CD44+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. *Oncogene* 2006;25:1696–1708. [PubMed: 16449977]
- Perez-Losada J, Balmain A. Stem-cell hierarchy in skin cancer. *Nat Rev Cancer* 2003;3:434–443. [PubMed: 12778133]
- Peters B, Kirfel J, Bussow H, Vidal M, Magin TM. Complete cytolysis and neonatal lethality in keratin 5 knockout mice reveal its fundamental role in skin integrity and in epidermolysis bullosa simplex. *Mol Biol Cell* 2001;12:1775–1789. [PubMed: 11408584]
- Pierce AM, Schneider-Broussard R, Gimenez-Conti IB, Russell JL, Conti CJ, Johnson DG. E2F1 has both oncogenic and tumor-suppressive properties in a transgenic model. *Mol Cell Biol* 1999;19:6408–6414. [PubMed: 10454586]
- Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, Weissman IL, Clarke MF, Ailles LE. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci U S A* 2007;104:973–978. [PubMed: 17210912]
- Ramirez A, Bravo A, Jorcano JL, Vidal M. Sequences 5' of the bovine keratin 5 gene direct tissue- and cell-type-specific expression of a lacZ gene in the adult and during development. *Differentiation* 1994;58:53–64. [PubMed: 7532601]
- Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414:105–111. [PubMed: 11689955]
- Robitaille H, Proulx R, Robitaille K, Blouin R, Germain L. The mitogen-activated protein kinase kinase dual leucine zipper-bearing kinase (DLK) acts as a key regulator of keratinocyte terminal differentiation. *J Biol Chem* 2005;280:12732–12741. [PubMed: 15695824]
- Ruiz S, Santos M, Segrelles C, Leis H, Jorcano JL, Berns A, Paramio JM, Vooijs M. Unique and overlapping functions of pRb and p107 in the control of proliferation and differentiation in epidermis. *Development* 2004;131:2737–2748. [PubMed: 15148303]
- Sawicki JA, Rothman CJ. Evidence for stem cells in cultures of mouse prostate epithelial cells. *Prostate* 2002;50:46–53. [PubMed: 11757035]
- Schuilenga-Hut PH, Vlies P, Jonkman MF, Waanders E, Buys CH, Scheffer H. Mutation analysis of the entire keratin 5 and 14 genes in patients with epidermolysis bullosa simplex and identification of novel mutations. *Hum Mutat* 2003;21:447. [PubMed: 12655565]
- Til HP, Woutersen RA, Feron VJ, Clary JJ. Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking-water study in rats. *Food Chem Toxicol* 1988;26:447–452. [PubMed: 3391468]
- Tumbar T, Guasch G, Greco V, Blanpain C, Lowry WE, Rendl M, Fuchs E. Defining the epithelial stem cell niche in skin. *Science* 2004;303:359–363. [PubMed: 14671312]

Figure 1.

BK5-T/t construct and characterization. (A) Schematic of the transgene construct. Top, the T open reading frame contains two exons and the t is transcribed in frame with the first exon of T antigen but terminates in the intron. The whole T/t coding region was amplified by PCR using the T antigen forward (For) and reverse (Rev) primers that contained the Not I and Nhe I restriction sites at the respective 5' ends. Bottom, the Not I/Nhe I fragment was cloned in the pBK5.197 vector, which contains the BK5 promoter, the rabbit β-globin second intron (which has been shown to increase the transgene expression, Ramírez et al., 1994) and the β-globin/SV40 poly A tail. The 9.5-kb fragment was purified and used for in vitro characterization and transgenesis studies. (B) NHP6 cells were transfected with the BK5-T/t Kpn I fragment and 48 h later, cells were stained for T (red) and counterstained using DAPI (blue). Most transfected cells show nuclear localization (arrowhead) although some cells show staining throughout the cell (arrow). Staining with the MOPC21 control monoclonal antibody did not reveal any specific labeling (data not shown). (C-D) NHP6 cells were transfected with the BK5-T/t Kpn I fragment and 72 h later, pulsed with BrdU. Four h later cells were stained for BrdU. Arrows indicate the two cells double positive for BrdU and T (note the red and green colors) and arrowhead indicates an untransfected BrdU⁺ cell. Shown in (D) is quantification of BrdU⁺ cells in mock-transfected (CTL) and BK5-T/t-transfected (LT) NHP6 cells. The numbers in the parentheses are cells counted (*P < 0.01, Chi-squared Test).

Figure 2.

Representative IHC images of T and CK5 staining in transgenics. **(A)** Examples of E9.5 transgenic embryo tissues including surface ectoderm (a-c), first branchial arch (d-f), olfactory placode (g-i), and limb bud (j-l). **(B)** Examples of E15 transgenic embryo tissues including surface ectoderm (a-c), forelimb (d-f), esophagus (g-i) and forestomach (j-l). **(C)** Examples of P1 transgenic tissues including skin (a-c), whisker follicle (d-f), forestomach (g-i), and trachea (j-l). Serial sections were stained for CK5 or T and representative images of both low (i.e., 100 $\times$) and high-power (i.e., 400 $\times$) magnifications were shown.

Figure 3.

Histological analyses of tongue epithelium (A and C) and palate mucosa (B and D). (A) Representative images of tongue from E20-P18 WT and transgenic (Tg) mice. Note the age-related maturation in filiform papillae in WT tongue that is lacking in the Tg tongue. (B) Representative images of palate mucosa from E20-P18 WT and Tg mice. For A and B, IHC revealed specific nuclear T staining in the tongue (A) and palate (B) epithelium (not shown). (C) Quantification of (dorsal) tongue epithelium thickness. For WT tongues, the inter-papilla epithelial thickness was measured. Six sections from each Tg and the age-matched WT animal were used to measure tongue epithelium thickness (7 fields with a total of 25 measurements per section) using a micrometer under a light microscope. (Ca) The E20 Tg tongue epithelium

is significantly thicker than the WT counterpart ($P < 0.0001$; Student's t -test). (Cb) Quantification of tongue epithelial thickness in E20 – P18 WT and Tg animals. Bars represent the mean $\pm$ SEM. (Cc) Quantification of Ki67-positive cells in WT and Tg tongue epithelia. Only the Ki67-positive cells in the basal layer were quantified and compared. The total numbers of cells counted were indicated. $*P < 0.01$ (Chi-squared test). (D) Quantitative analyses of palate mucosa thickness (a and b) and Ki67+ positive cells (c). All measurements and statistical comparisons were performed as described in C (a-c). (d) Representative images of E20 WT and transgenic palate mucosa stained for Ki67. Note the markedly increased Ki67-positive cells in the transgenic palate across both basal and suprabasal layers.

Figure 4.

Aberrant differentiation in the BK5-T/t transgenic tongue epithelium, palate mucosa, and taste bud. Shown are representative images of P1 (A) and P18 (B) WT or transgenic (tg) tongue (c-f) and palate (g-j) stained for CK13 or CK5. The original magnifications for panels a and b are 40x and for all the rest, 400x. In (C), one representative fungiform papilla containing a taste bud is shown for P18 WT or transgenic (tg). (a-d), HE staining; (e-h) CK13 staining.

Figure 5. Epidermal hyperplasia and aberrant differentiation in the transgenic mouse. (**A** and **B**) Representative P1 (**A**; dorsal skin in the back shoulder region) and P18 (**B**; scalp) WT and transgenic (tg) skin stained for HE (a-d) or CK10 (e-h). (**C** and **D**) Quantification of P1 (**C**) and P18 (**D**) WT and transgenic (tg) epidermal thickness. Four non-consecutive sections each from WT and Tg mice were measured for epidermal thickness and data pooled from 7–10 fields/section (50 measurements per section). The difference between the Tg and WT epidermis is statistically significant for both P1 and P18 comparisons ($P < 0.001$, Student's *t*-test).

Table 1
T protein expression in BK5-T/t transgenic tissues/organs examined at different stages

Stage	CK5+ tissues/organs			CK5- tissues/organs		
	Tissues	T	CK5	Tissues	T	CK5
E9.5	surface ectoderm	+	+	primitive neuroectodermal tissue around the fourth ventricle	+	-
	limb bud epithelium	+	+			
	olfactory placode	+	+			
	first branchial arch	+	+			
	surface ectoderm	+	+	cranial mesenchyme	+	-
E13	olfactory epithelium	+	+			
	first pharyngeal pouch	+	+			
	surface ectoderm, forelimb	+	+	cranial mesenchyme	+	-
E15	primordia of follicles of vibrissae	+	+			
	corneal epithelium	+	+			
	olfactory epithelium	+	+			
	tongue epithelium	+	+			
	esophagus, stomach	+	+			
	thymus	+	+			
	epidermis, whisker vibrissa	+	+	cranial mesenchyme	+	-
	tongue and palate epithelium, enamel	+	+	midbrain	+	-
	nasal epithelium, trachea	+	+			
	Eustachian tube	+	+			
E20	submandibular gland	+	+			
	thymus	+	+			
	esophagus, forestomach	+	+			
	pancreas (ductal epithelium)	+	+			
	ureter/renal pelvis, bladder	+	+			
	urethra, penis	+	+			
	epidermis, whisker	+	+	midbrain	+	-
	tongue and palate epithelium, enamel	+	+	lung	+	-
	nasal epithelium, trachea	+	+			

Stage	CK5+ tissues/organs		CK5- tissues/organs	
	Tissues	T	Tissues	T
P2	Eustachian tube	+		+
	submandibular gland	+		+
	thymus	+		+
	esophagus, forestomach	+		+
	pancreas (ductal epithelium)	+		+
	ureter/renal pelvis, bladder	+		+
	urethra, penis	+		+
	skin, whisker	+		+
	tongue and palate epithelium, enamel	+		+
	nasal epithelium, trachea	+		+
	Eustachian tube	+		+
	submandibular gland	+		+
	thymus	+		+
	esophagus, forestomach	+		+
P18	pancreas (ductal epithelium)	+		+
	ureter/renal pelvis	+		+
	skin, whisker vibrissa	+		+
	tongue and palate epithelium, enamel	+		+
	nasal epithelium, trachea	+		+
	bronchus	+		+
	submandibular gland	+		+
	thymus	+		+
	esophagus, forestomach	+		+
	pancreas (ductal epithelium)	+		+
	ureter/renal pelvis, bladder	+		+

T staining was carried out on 4- μ m sections by using the T-specific mAb. IHC of CK5 was performed by using a rabbit polyclonal antibody that recognizes the C-terminus of mouse keratin 5 protein.