Commentary

Keratins: Markers of cell differentiation or regulators of cell differentiation?

Keratins are epithelia-specific intermediate filament proteins, which are expressed in a tissue-specific manner (Moll *et al* 1982). Around 50 keratin genes have been discovered across the species. Keratins have been classified according to their molecular weights and isoelectric points. They have been further subdivided into two subtypes, type I, which are acidic and have low molecular weights, and type II, which are basic or neutral and have high molecular weights. Keratins are obligatory heteropolymers, and the expression of at least one member of each subfamily is essential for proper filament formation (Coulombe and Omary 2002). The transcription factors regulating keratin gene expression include members of the AP1 and SP1 families (for keratin 16; Wang and Chang 2003), C/EBP and AP-2 (for keratin 10; Maytin *et al* 1999), and AP1 and ETS family members (keratin 8/18; Hendrix *et al* 1996). However, these transcription factors do not govern their tissue-specific expression. Keratins, like all other intermediate filaments, form are highly insoluble structures and require conditions such as 8–9.5 M urea or 1% sodium dodecyl sulphate with boiling for their dissolution. Because of their highly insoluble nature, they were thought to be static structures having only structural functions. It is now clear that keratin filaments are highly dynamic in nature and post-translational modifications such as phosphorylation contribute to their dynamic nature (Omary *et al* 2006). Their role in the structural integrity of cells was emphasized by the appearance of keratinopathies, which have been shown to be the direct result of single-point mutations in keratin genes (Coulombe and Omary 2002). Some of the severe skin disorders which result from point mutations include epidermolysis bullosa simplex (mutations in keratin 5/14 genes), epidermolytic hyperkeratosis (mutations in keratin 1/10 genes) and epidermolytic palmoplantar keratoderma (mutations in the keratin 9 gene). The point mutations in these keratin genes result in the formation of abnormal keratin filaments or protein aggregates, and lead to malfunctioning. In these diseases, the skin becomes scaly and even the slightest insult to the skin results in boils and erythema. Similar phenotypes have been observed in respective transgenic mice (Magin *et al* 2000). Mutations in the K8/18 genes have also been associated with diseases such as acute pancreatitis and liver cirrhosis (Omary *et al* 2004).

The effects of mutation in a particular keratin are more deleterious due to protein aggregation rather than the absence of that keratin, probably because it would also result in defective interactions with membrane proteins, affecting their function of imparting structural resilience. Similar effects have been observed as a result of mutations in keratin-associated proteins such as plectin and desmoplakin (Omary *et al* 2004).

Well-established structural functions alone cannot explain the diversity and dynamic nature of keratin filaments. Several questions pertaining to the multiplicity of keratins and their probable tissue function remain unanswered. These unresolved issues include:

(i) If keratins have only structural function, what is the need for such a variety of keratins?
(ii) Why do they exhibit tissue-specific expression?
(iii) What are the factors regulating their tissue-specific expression?
(iv) Do keratins have regulatory roles?

Studies conducted to understand the multiple functions of keratins have indicated that keratins modulate processes such as osmolarity (Toivola *et al* 2004), apoptosis (Gilbert *et al* 2004) and regulate protein synthesis (Kim *et al* 2006). These processes are regulated by modulation of signalling pathways. Kim *et al.* have demonstrated the role of keratin in maintenance of homeostasis and regulation of protein synthesis. Expression of Kb6a, Kb6b and keratin 17 was found to be upregulated in cells close to the

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Table 1. Signalling pathways modulated by keratins

Keratin	Pathway	Reference
K8/18	Fas signalling/apoptosis	Gilbert *et al* 2001
K8/18	TNFα signalling/apoptosis	Caulin *et al* 2000
K14	TNFα signalling/apoptosis	Inada *et al* 2001
K17	TNFα signalling/apoptosis	Tong *et al* 2006
K 10	TNFα signalling/apoptosis	Chen *et al* 2006
K18	Cell cycle	Kuno *et al* 2002
K10	PI-3K pathway/cell proliferation	Paramio *et al* 2001
K17	mTOR pathway/ protein synthesis	Kim *et al* 2006
K10	Notch signalling pathway/ differentiation	Santos *et al* 2005
K6/16	Cell proliferation	Paladini *et al* 1998

wound. Unlike keratin 17 knockout, double knockouts mice of Kb6a and Kb6b did not exhibit delayed wound closure. In a keratin 17 -/- background, the cells close to the wound were smaller and not growing properly. Translation regulated by mTOR was found to be defective. Here, keratin 17 was shown to regulate translation in a 14-3-3σ dependent association, as mutation of two residues important for 14-3-3σ binding did not result in increased translation. Some of the signalling pathways modulated by keratins are shown in table 1. Other functions of keratins, such as their role in cell differentiation and transformation, are still emerging. However, the factors regulating tissue-specific expression of the keratins –themselves are still largely unknown (Magin *et al* 2005). Table 2 contains a compilation of the expression patterns and related information of important keratins**.** A number of studies have demonstrated differentiation-dependent expression of keratins. Thus, they are being routinely used as markers of cell differentiation (Franke *et al* 1982; Clausen *et al* 1986; Swaf *et al* 1990). For example, keratin 6/16 are associated with cell proliferation while keratin 1/10 are markers of cell differentiation.

In stratified epithelia, the cells of the basal layer are highly proliferating and express keratin 5/14. As they migrate into the upper layer, they become more differentiated. Cells of the uppermost layer are terminally differentiated cells that express keratin 4/13 or keratin 1/10, depending upon the differentiation state of the tissue. For example, non-keratinizing epithelia such as the buccal mucosa and soft palate express keratin 4/13 while keratinizing epithelia such as the skin and tongue express keratin 1/10. Alterations in differentiation-related keratin have been seen after malignant transformation. Epithelial cells have also been shown to undergo phenotypic changes possibly due to cellular de-differentiation and concomitant changes in the keratin profile; the appearance of vimentin has also been documented (Hendrix *et al* 1996)

Keratin 6 and 16 are constitutively expressed at low levels in a number of stratified epithelia including palmar and plantar epidermis, tongue, oral mucosa and the outer root sheath of the hair follicle. These keratins are not present in normal interfollicular epidermis, but their expression is rapidly induced in suprabasal cells located at the edges of wounds. In addition, keratin 6 and 16 are also expressed in stratified epithelia featuring hyperproliferation or abnormal differentiation, such as psoriasis and cancer (McGowan and Coulombe 1998).

The keratin pair of 1/10 is normally expressed by the suprabasal layers of keratinizing stratified epithelia such as the epidermis, dorsal tongue, hard palate, etc, while non-keratinizing stratified epithelia such as that of the esophagus and buccal mucosa do not express this pair (Moll *et al* 1982). Keratin 1/10 is known to be a marker of cellular differentiation, and many well-differentiated squamous cell carcinomas (SCCs) derived from non-keratinizing stratified epithelia also express this keratin pair (Vaidya *et al* 1989; Vigneshwaran *et al* 1989).

One of the studies conducted to understand retinoic acid function indicated that retinoic acid deficiency led to changes in keratin expression, and these changes preceded histological alterations (Gijbels *et al* 1992). In our view, this was the first indication of keratins having some regulatory function with respect to cell differentiation. Further studies using *in vitro* tissue culture systems as well as transgenic and knockout mice support our view that keratins themselves regulate cell differentiation and are not merely markers of cell differentiation.

Table 2. Expression pattern and related information of some important keratins

Keratin	Normal expression	@ Knockout phenotype; # related human pathology	Comments	References
K1	Epidermis, foot sole epidermis, anal canal epithelium, portio uteri (exocervix)		Marker of cell differentiation	Moll *et al* 1982
K5	Epidermis, foot sole epidermis, outer root sheath of hair follicle, sebaceous gland, cornea, portio uteri (exocervix), tongue, epiglottis, oesophageal, anal canal, tracheal and amnion epithelium, apocrine gland from axilla (acini), eccrine sweat gland (total), mammary gland ducts	@Extensive skin blistering, cytolysis of basal cells; # Epidermolysis bullosa simplex	5/14 form basic expression pair for all stratified and transitional epithelia. Expression predominantly in basal layer	Moll *et al* 1982; Magin *et al* 2005
K6	Tongue, epiglottis, anal canal epithelium, outer root sheath of hair follicle, eccrine sweat gland (total), foot sole epidermis		Marker of cell proliferation	Moll *et al* 1982
K7	Apocrine gland from axilla (acini), eccrine sweat gland (total), mammary gland ducts, transitional epithelium of bladder, gall bladder epithelium.			Moll *et al* 1982
K8	Hepatocytes, colon, small intestine (mucosa), transitional epithelium of bladder, amnion, trachea, gall bladder epithelium, mammary gland ducts, exocrine sweat gland (total), apocrine gland from axilla (acini)	@Embryonic lethal, mice of different genetic background survive (FVB/N) and exhibit colorectal hyperplasia, colitis, rectal prolapse, predisposition to liver injury	Role in cell transformation/ differentiation	Moll *et al* 1982; Magin *et al* 2005
K10	Suprabasal epidermis, foot sole epidermis, anal canal epithelium	@ Acanthosis, hyperproliferation of basal cells, hyperkeratosis; # Non-epidermolytic hyperkeratosis	Marker of cell differentiation	Moll *et al* 1982; Magin *et al* 2005
K14	Epidermis, foot sole epidermis, outer root sheath of hair follicle, sebaceous gland, anal canal epithelium	@ Extensive skin blistering, cytolysis of basal cells; # Epidermolysis bullosa simplex	5/14 form basic expression pair for all stratified and transitional epithelia. Expression predominantly in basal layer	Moll *et al* 1982; Magin *et al* 2005
K17	Outer root sheath of hair follicles, amnion and trachelal epithelium, mammary gland ducts	@ Alopecia; # Pachyonychia congenita	Regulates growth and proteins synthesis.	Magin *et al* 2005; Kim *et al* 2006
K18	Hepatocytes, colon, small intestine (mucosa), gall bladder epithelium, excrine sweat glands (total)	@ Mild liver pathology	Role in cell transformation/ differentiation	Moll *et al* 1982; Magin *et al* 2005

Directed expression of keratin 16 instead of keratin 14 in the progenitor stem cells of newborn mice resulted in abnormal cell proliferation of the suprabasal cells of the skin. This suggested that keratin 16 may carry signals for cell proliferation (Paladini *et al* 1998). In another study, increased keratin 16 gene expression was seen in response to epidermal growth factor (EGF) treatment in HaCaT cells. It was also shown that Sp1 acted synergistically with c-Jun to activate keratin 16 gene expression (Wang and Chang 2003).

Downregulation of keratin 10 in skin papillomas induced in mice by the 7,12-dimethylbenz[a]anth racene (DMBA) – 12-0-tetradecanoylphorbol-13-acetate (TPA) protocol, and its subsequent absence in carcinomas has also been demonstrated (Roop *et al* 1988). Ablation of the keratin 10 gene from the suprabasal layers of the skin has been shown to lead to hyperproliferation of basal cells, induction of c-myc, cyclin D1, 14-3-3σ, keratin 6 and keratin 16 (Reichelt *et al* 2002). It has also been shown that this results in higher turnover and early differentiation of these cells. Increased expression of keratin 10 in the basal layer of epidermis in mice led to a hypoplastic and hyperkeratotic epidermis, due to a dramatic decrease in skin keratinocyte proliferation in association with the inhibition of Akt and PKC ζ activities (Santos *et al* 2002). In another study, expression of keratin 10 in the thymic epithelium of mice resulted in abnormal cell differentiation and altered notch signalling not only in the thymic epithelium but also in thymocytes (Santos *et al* 2005). In contrast, it has recently been demonstrated that chimeric keratin 10 end domains fused to keratin 14 rod domains in the basal layer of the epidermis in mice are responsible for resistance to TNF-α mediated apoptosis, and it temporarily/partially inhibits apoptosis for timely differentiation of keratinocytes (Chen *et al* 2006). These authors suggest that the earlier findings of Santos *et al* were merely the result of over- expression of keratin 10. Although the effects of ectopic expression of keratin 10 are controversial, the fact remains that keratins do have a regulatory role in controlling differentiation/proliferation. Thus, keratin 16 and keratin 10, which are markers of cell proliferation and cell differentiation, respectively, themselves appear to regulate cell proliferation/ differentiation.

Another interesting pair is keratin 8/18. This keratin pair is normally expressed by simple epithelia such as those of the liver and pancreas. In mixed epithelia such as those of the breast and lung, this pair is expressed in the suprabasal layers and is associated with normal differentiation. The keratin 8/18 pair also appears to regulate many tissue-specific functions in simple epithelia, and modifies stratified epithelial function when expressed aberrantly. For example, certain members of the 14-3-3 family and heat shock proteins are known to associate with keratin 18 in a phosphorylation–dependent manner, probably resulting in the modification of their function (Ku *et al* 1996). Keratin 8/18 also resists receptor-mediated apoptosis in hepatocytes (Gilbert *et al* 2001). This keratin pair along with vimentin imparts invasive, metastatic and drug-resistant properties to cells (Hendrix *et al* 1996).

Aberrant expression of this keratin pair is observed in many squamous cell carcinomas (Vaidya *et al* 1989, 1996, 1998; Ranganathan *et al* 2006). Our group has demonstrated that aberrant keratin 8 and 18 expression leads to malignant transformation of stratified epithelial cells. Transfection of keratin 8 in an immortalized but non–transformed stratified epithelial cell line resulted in keratin 8/18 filament formation and subsequent cell transformation (Raul *et al* 2004). Expression of keratin 8 gene in the skin of transgenic mice resulted in the formation of keratin 8/14 filaments and hyperproliferation of epidermal cells. The conversion rate of papillomas to carcinomas on treatment with chemical carcinogens also increased in transgenic animals. Thus it appears that aberrant keratin 8 expression in the skin leads to deregulation of cell differentiation (Casanova *et al* 2004). Another group documented opposite results when keratin 18 was transfected in MDA-MB-231 cells. This cell line derived from transformed breast epithelium, exhibits much lower expression of the keratin 8/18 pair. Upon transfection of keratin 18, vimentin was downregulated and keratin 8 expression was induced. This resulted in appearance of a less invasive and more differentiated phenotype (Buhler *et al* 2005). These differential effects of ectopic keratin 8/18 expression can be explained by the fact that this pair is expressed by the suprabasal layer of mixed epithelia and is differentiation-associated. In stratified epithelium, aberrant expression of this keratin pair results in deregulation of its normal differentiation programme.

The molecular events modulating cell differentiation in response to either abnormal expression in stratified epithelia (where this pair is not normally found), or their lowered expression in mixed epithelia (where it is normally found) have not been elucidated yet. Some of these issues are being currently addressed in our laboratory.

Evidence from the available literature on transgenic and knockout animal studies compels us to state that keratins should not be considered merely as markers but also as "regulators" of differentiation. However, the intricate molecular mechanisms modulated/regulated by keratins as well as the regulation of their own expression pattern in the tissue context are yet to be unravelled and warrant further investigation.

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Milind M Vaidya* and Deepak Kanojia
KS 110 - 111, Vaidya Lab,
Cancer Research Institute,
Advanced Centre for Treatment Research and Education
in Cancer, Tata Memorial Centre, Kharghar,
Navi Mumbai, Maharashtra 410 210, India
**(Email, mvaidya@actrec.gov.in)*

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