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Homeobox Genes, Fetal Wound Healing, and Skin Regional Specificity

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Why do adult wounds heal with scars but fetal wounds heal without scars? Why do fetal tissues regenerate while adult tissues only repair damage? Why do some parts of adult skin produce scars more than other parts? Why in some genetic diseases or infectious diseases, are only particular skin regions affected, forming specific anatomical patterns? How are these temporal and spatial differences in the skin regions established?

The ability of a targeted tissue to respond to stimulation is what embryologists call ‘competence’. It can refer to an ability to form a particular type of skin appendage, to regenerate functional tissues, to heal without forming scars, or to form excessive scars in response to injury. What is the molecular basis of ‘competence’? Progress in biomedical research has allowed us to begin to explore the molecular mechanisms behind competence. Since fetal and adult cells respond to the same external stimuli or microenvironments differently, the difference must be intrinsic to the cells. It can be in the form of membrane receptors, cytoplasmic signal relaying molecules or the composition of transcription factors available in the nucleus of responding cells. As developmental programs unfold, cells and tissues in different body regions are specified differently. With different developmental histories and different molecular compositions or ‘memories’, their responses to the same stimuli begin to diverge.

Homeobox genes are the leading candidate molecules involved because of their demonstrated roles in morphogenesis (Gehring, 1987; Scott and Goldsmith, 1993). Homeobox genes are transcription factors that share homeobox domains, which bind the enhancers of downstream genes and regulate their expression. Homeobox genes are regulated in such unique ways that they themselves are expressed in specific anatomical regions, such as in the distal but not proximal limb buds (e.g. Hoxd13), or in forelimb but not hindlimb buds (e.g. Tbx5). In the homeobox gene category, Hox genes are the major family, but there are also Dlx, Msx, T box, Prx, etc. family members. Hox genes are expressed in colinearity (nested expression pattern, and the sequence corresponds to their positions on the chromosome) along the body axis, and later along the limb axis. They are involved in the specification of different morphologies of vertebrae, and later the shape of limb skeletal elements. Knocking out Dlx 5 and 6 causes the formation of mirror-imaged double upper jaws, including the re-specification of the lower jaw dermis to form vibrissae (Shigetani *et al*, 2002). Mis-expression of Tbox 4 and 5 can transform a chicken wing to

become a leg and vice versa, including the change of dermis to form scales or feathers (Rodriguez-Esteban *et al*, 1999). The presence of Msx 1 was shown to be associated with the competence to regenerate digits (Reginelli *et al*, 1995).

In the skin, classical epithelial-mesenchymal recombination experiments have demonstrated that much of the skin regional specificity is determined by the dermis (Sengel, 1976). Along this line of research, chicken skin is an excellent model because of its distinct characteristics (feathers vs. scales, bilateral vs. radial feather symmetries) and accessibility to experimental embryological approaches. Hox genes were shown to form different sloped microgradients in feather buds from different skin regions (Chuong *et al*, 1990) and the skin Hox codes were hypothesized to be the basis for regional specificity of the skin (Chuong, 1993). A more systematic survey of Hox expression on developing chicken skin was recently performed, and these authors report intriguing findings that there are both colinear and noncolinear expression patterns. Some Hox genes show regional restricted expression (Hox a7, b4, and c8), while others are expressed later in development, concomitantly and unrestrictively (H ox a11, c6, d4, d13) (Reid and Gaunt, 2002). Thus there maybe more than one epoch of Hox function: the first epoch for regional specificity determination, and the second epoch for regulating intra-appendageal morphogenesis. The whole picture has not emerged yet. When retinoic acid was added to transform developing scales into feathers, Hox d13, originally expressed in the plantar dermis of the foot, was suppressed. This is consistent with the hypothesis that homeobox genes are involved in specifying regional identity of skin territories (Kanzler *et al*, 1997).

In mice, Hox expressions in embryonic skin (Detmer *et al*, 1993) and in cycling hair follicles (Packer *et al*, 2002) have been determined for some family members. Mice with Lac Z driven by the enhancer region of Hox 3.1 showed a remarkable regional expression pattern on the skin (Bieberich *et al*, 1991). However, these studies are limited to a few Hox genes and a systematic mapping of Hox gene expression in the whole skin region has not yet been accomplished. While many Hox related mutants were generated to help analyze the roles of Hox genes in skeleto-genesis, the phenotypes in the skin of these mutants have not been obvious. In a Hox c13 mutant, there is an apparent alopecia phenotype. However, analysis showed that the defect appears to be a problem of hair differentiation, not morphogenesis (Godwin and Capecchi, 1998).

In humans, expression of both Hox and non-Hox genes (e.g. Msx, Prx) in fetal and adult skin were studied. Higher and wider expression of these genes in fetal vs. adult skin were observed (Stelnicki *et al*, 1997; 1998a, b). Hox genes tend to be in keratinocytes and Prx genes tend to be in dermal fibroblasts. Furthermore, using immuno-histochemical studies, this group showed that Hox B6 is regulated at the subcellular level: a homeodomain-truncated cytoplasmic form is dominant in the fetal epidermis, but a homeodomain-containing form is located in the nucleus and prevails in normal adult skin (Komuves *et al*, 2000). They did not report apparent regional specific expression patterns (in contrast to Chang *et al*, 2002; see below), but this could be due to difficulties in obtaining specimens for a more complete survey. They studied the roles of Hox genes more in terms of growth control. HOX B4 is associated with proliferative status, including psoriasis and basal cell carcinoma. Forced expression of HOX B4 in keratinocytes leads to increased proliferation

and down regulation of integrin alpha 2 and CD44 adhesion molecules (Komuves *et al*, 2002). Association of Hox genes with keratinocyte differentiation has also been suggested. Indeed some hair keratin and transglutaminase were shown to be the downstream targets of Hox c13 and Hox a7, respectively (Tkatchenko *et al*, 2001; La Celle and Polakowska 2001; Stelnicki *et al*, 1997).

In this issue, White *et al* (2002) tackle further the roles of homeobox genes in scarless fetal wound healing. It has been the premise of plastic surgeons that if they can learn how fetal cells handle wound healing, they can apply similar principles to adult wound healing and manage wounds better (Peled *et al*, 2000). To this end, they have been searching for key molecules that can distinguish between adult and fetal fibroblasts to make them behave differently. Members of the TGF beta pathway, matrix metallo-proteinase (MMP), etc. have been associated with this role (Soo *et al*, 2000). In the earlier work, homeobox genes Prx-2 and Hox b13 were shown to be expressed in high levels in fetal dermal fibroblasts. These genes were further induced during fetal wound healing. In contrast, in adult fibroblasts their levels were low and not inducible in response to wounding (Stelnicki *et al*, 1998a). In the current paper, taking advantage of the newly available Prx2 knockout mice, this group further compared the *in vitro* behavior of fetal and adult fibroblasts derived from Prx2 −/− and control mouse skin. They showed that a lack of the Prx2 gene influenced the expression of pro-MMP2 and increased the production of hyaluronic acid, but did not affect cell proliferation, cell-substrate adhesion, or the production of collagen. The absence of Prx2 did alter the ability of fetal fibroblasts to organize extracellular matrix in a three-dimensional collagen lattice.

The paper did not establish a causal relationship firmly by suppressing Prx2 from normal fetal fibroblasts, or by ectopically expressing Prx2 in adult Prx2−/− fibroblasts. Nor did they explore the *in vivo* wound healing responses of fetal and adult Prx2 knockout mice. Obviously, these are interesting future experiments. They may also try to establish a link to TGF beta and other factors known to be involved in the behavior of fetal fibroblasts. This paper does demonstrate that several fetal fibroblast behaviors are due to one single homeobox gene, Prx2. It represents a significant step toward identifying the molecular basis of competence.

With a different approach, a paper with impact has just appeared. Using cDNA microarrays to profile 36,000 genes, Chang *et al* (2002) found that human dermal fibroblasts from different body regions and different ages express distinct sets of genes which they term ‘topographic differentiation’. In contrast to Stelnicki *et al* (1998b) that report no detection of Hox genes in adult dermis, nor regional differences in the epidermis that express Hox genes, Chang and colleagues found distinct Hox gene expression patterns in different fibroblasts obtained from different skin regions of the adult (gum, arm, abdomen, thigh, toe, foreskin, etc.). This work is not without problems. The experimental design is not as comprehensive as it could be, probably also due to practical issues in obtaining human specimens. However, it elegantly highlights the immense power of microarray technology, and points out what may be possible in the coming new era of research. While genetic differences, through inheritance or somatic mutations, undoubtedly play a role in the differences observed in temporally and regionally different specimens, here we shall focus our attention at the level

of developmental and regional differences within the same normal individual, whose cells presumably share the same genome but express different transcriptomes or proteomes.

Studies on the roles of homeobox genes in skeletal patterning have made remarkable progress due to the mouse model. Using ingenious designs of genetically engineered mice and careful analysis of distinct skeletal phenotypes, new ground has been broken to establish the molecular basis of colinearity and enhancer control of regional specificity(Kmita *et al*, 2002). Can we raise the research of skin temporal and regional specificity to the same level of sophistication? One practical problem is that the mouse is not an ideal model for this purpose because mouse skin lacks clear regional specificity as is found in humans or chickens. However, some work still managed to address the dorso-ventral polarity of paws, including the formation of hairs, claws and sweat glands (Loomis *et al*, 1996). We may need to develop protocols to look into the skin phenotypes in much more depth to reveal differences in temporal control and regional specificity. We can use the many available genetically engineered mice whose skin phenotypes may have been overlooked. In fact, the current White *et al* (2002) paper is one positive example that analyzes the available Prx2 −/− mouse further. Chicken skin has much more distinct phenotypes and retroviral technology can now be applied routinely to chicken skin to alter gene expression, make psuedo-transgenic skin appendages, and test various hypotheses (Yu *et al*, 2002). However, real transgenic chicken technology is not yet available, and analysis at the enhancer level lag behind. Humans also offer remarkable temporal and spatial skin regional specificity. The knowledge of clinical genetics adds to this treasure (Happle, 1995). However, research on human skin has been compromised due to the limited availability of specimens and accessibility for experimentation, but advances in the human genome project and microarray gene profiling technology (as shown in Chang *et al*, 2002) with small amounts of materials may change this situation rapidly. It may take the combination of these different models for us to gain new levels of understanding. While this is still a young field, the time is ripe for new discoveries to be made.

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