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The Focal Nature of Darier's Disease Lesions: Calcium Pumps, Stress, and Mutation?

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Haploinsufficiency of the *ATP2A2* gene product, SERCA2, underlies most cases of Darier's disease. Sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase isoform 2 (SERCA2) is an intracellular Ca^{2+} pump that replenishes ER Ca^{2+}, and it seems likely that the disease manifests in stress-induced lesions because SERCA levels become limiting as extra demands are made on the pump in times of stress. However, Müller and colleagues (2006) present a radical new proposal invoking somatic mutation as the basis for Darier lesions. Using a novel animal model for depleted keratinocyte SERCA-gated Ca^{2+} stores, the authors show that keratinocytes from Darier-like lesions retain their distinctive phenotype after culture, suggesting heritable defects. Mechanistically linking stress, calcium levels, mutation, and disease pathogenesis is complicated, and the proposal is likely to be controversial. However, recent reports of age- and stress-dependent tumor formation in the mouse model for SERCA2 haploinsufficiency (*ATP2A2* heterozygous mouse) support the proposal that deficiency in SERCA-gated ER Ca^{2+} replenishment may be linked to mutation accumulation.

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Mutations in intracellular calcium pump genes underlie the genetic skin diseases Darier's disease (DD) and Hailey-Hailey disease (Sakuntabhai *et al.*, 1999; Hu *et al.*, 2000). These genodermatoses are characterized by defects in keratinocyte adhesion and, in the case of DD, keratinization defects. Mutation in the *ATP2A2* gene, which encodes sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase isoform 2 (SERCA2), an adenosine triphosphate-dependent calcium pump that transfers calcium from the cytosol to the calcium-rich lumen of the endoplasmic reticulum (ER), is the common mutation underlying DD (Foggia and Hovnanian, 2004). The SERCA2 pump influences the formation of calcium oscillations, regulates return to resting cytoplasmic calcium concentrations after signal-induced ER calcium mobilization, and maintains the calcium-rich environment of the ER lumen. Defects in pump action may affect a wide range of signaling events, as well as post-translational protein processing and chaperone-mediated trafficking.

Although the mechanism linking intracellular calcium regulation and the DD-associated hyperkeratinization is unknown, its basis in an internal calcium pump defect seems uncontroversial, as a role for calcium mobilization from internal stores during keratinocyte terminal differentiation is expected (Bikle *et al.*, 2001). The DD adhesion defects have been histologically attributed to desmosomal disruption and internalization. The relationship between desmosomal protein trafficking and the intracellular calcium stores is demonstrated by use of the drug thapsigargin, which specifically inhibits SERCA pumps, mimicking the defect in DD keratinocytes. Thapsigargin-induced depletion of intracellular ER calcium stores inhibits trafficking of desmosomal components, principally desmoplakin, to the cell membrane (Stuart *et al.*, 1996; Dhitavat *et al.*, 2003), and desmoplakin trafficking is defective in cultured DD keratinocytes (Dhitavat *et al.*, 2003). Given the dynamic nature of desmosomes during differentiation, it seems probable that defective trafficking of components contributes to the acantholysis (intercellular splitting) that is characteristic of DD.

Whatever the precise disease pathomechanism, the discovery that dysregulation of a fundamental piece of cellular machinery, such as an intracellular calcium pump, underlies DD prompts the question of why the keratinization and adhesion defects are lesional rather than widespread. The answer is probably based in the finding that DD is a dominant disorder and that its dominance derives principally from haploinsufficiency (Foggia and Hovnanian, 2004). Reduced SERCA2 protein levels underlie most DD cases. SERCA2 levels conferred by one allele are probably sufficient under normal conditions, leading to the apparently normal phenotype of non-lesional skin. However, DD lesions tend to be triggered by stress (sweating, heat, UV light, mechanical irritation). Possibly, under stress conditions, the level of pump activity conferred by a single allele is insufficient to maintain normal keratinization and desmosome turnover (Foggia and Hovnanian, 2004).

A new, radical proposal by Müller and colleagues (2006, this issue) suggests that DD lesions are due to mutation. Müller and colleagues used a canine genetic model with skin lesions histologically similar to DD lesions and, intriguingly, depleted keratinocyte SERCA-gated calcium stores demonstrated by thapsigargin challenge. SERCA2 protein levels are not decreased in this model, and the mutational basis of the skin defect is unknown. However, this canine model provides an invaluable source of material for analysis of the effects

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of ER calcium store depletion on skin. The model may be particularly useful as SERCA2-null mice have been uninformative with respect to DD lesion pathology; the homozygous knockout is lethal, whereas the heterozygous mouse, which should phenocopy haploinsufficiency in human disease, does not develop Darier-type skin lesions (Prasad *et al.*, 2005). In addition, the authors point out that there is a concentration of studies on lesional human DD skin and a paucity of non-lesional analysis — perhaps not surprising given the ethical constraints on sampling normal skin in distressed patients.

The unexpected finding from the diseased canine model is that whereas cultured keratinocytes from non-lesional skin undergo normal differentiation, keratinocytes from lesions maintain their "lesional" phenotype (that is, display keratinization and adhesion defects) after passage in culture, suggesting permanent change as the basis for the lesion. Mutation is an obvious candidate for the permanent change in lesional keratinocytes.

...a radical new proposal invoking somatic mutation as the basis for Darier lesions

Because deficiency in SercA-gated calcium replenishment in non-lesional keratinocytes from the diseased dog did not produce major defects in keratinocyte steady-state adhesion and terminal differentiation, yet left the cells susceptible to formation of lesions, Müller and colleagues (2006) set out to find the primary physiological consequence of the defect in calcium store replenishment.

Both increased numbers of proliferative cells and enhanced apoptosis occur in non-lesional skin from the diseased dog as compared with wild-type skin. Later stages in terminal differentiation are unaffected, consistent with the "wild-type" morphology of non-lesional skin. Delayed exit from the cell cycle associated with delayed induction of the cyclin-dependent kinase inhibitor $p21^{WAF1}$ as keratinocytes enter terminal differentiation has been reported (Dotto, 1999). Induction of $p21^{WAF1}$ is associated with cell cycle withdrawal and onset of keratinocyte terminal differentiation (Dotto, 1999), and the inference is that defective mobilization of internal calcium or some other defect associated with ER store depletion leads to impaired regulation of $p21^{WAF1}$.

Müller and colleagues (2006) have developed a complex and ingenious argument linking these cell cycle changes with an increased, stress-associated mutation rate in DD. It is proposed that in the presence of stress or trauma a delay in cell cycle exit and impairment of $p21^{WAF1}$ upregulation are damaging, as they might increase the likelihood of cell cycle progression in mutated keratinocytes without repair. Hence, genetically based lesions would appear in skin subject to stress. In addition, it is speculated that ER calcium stores may be linked to regulation of the $p53/p21^{WAF1}$ checkpoint controls, which, when compromised, can lead to accumulation of secondary mutation.

Many aspects of this novel hypothesis may be controversial when related to DD. For example, is Darier lesion size compatible with clonal change? Is a mutational basis for Darier lesions compatible with remission? Though UV stress is associated with genomic damage, are some of the other types of stress and trauma associated with Darier lesions (microbial action in seborrheic regions, heat, mechanical friction) likely to lead to genomic damage? Is the focal nature of Darier lesions remarkable, given that the majority of skin genodermatoses manifest regionally? In addition, an important caveat associated with the primary observation of cell-heritable phenotypic change is that lesional keratinocyte cultures were from one papule from one of the diseased dogs, and verification in additional lesions and animals is desirable. However, this important observation of cell-heritable phenotypic change maintaining characteristics of Darier lesions does require explanation and further investigation. The hypothesis also generates many testable predictions.

A most interesting parallel to this proposal is the finding that, although mice heterozygous for the SERCA2 null allele do not form DD-type lesions, they have an increased tendency, with age, to form papillomas and squamous-cell carcinomas, particularly in areas subject to mechanical stress. Tumor formation occurs without loss of heterozygosity; that is, SERCA2 is still present at lower levels (Prasad *et al.*, 2005). Hence, deficiency in SERCA2 levels and consequent changes to calcium homeostasis render keratinocytes more susceptible to tumorigenesis in an additional model, lending authenticity to this radical proposal linking dysfunction of ER calcium stores with mutation accumulation in the presence of stress.

CONFLICT OF INTEREST

The author states no conflict of interest.

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