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Commentary

Lessons from the $\alpha 2$ Integrin Knockout Mouse

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The $\alpha 2\beta 1$ integrin (VLA-2), one of the first integrins to be identified and characterized, is a collagen receptor, although it can function as a dual collagen/laminin receptor on some cell types.[1] Numerous studies have implicated this integrin in a range of biological and pathobiological functions.[2,3] A more rigorous analysis of $\alpha 2\beta 1$ function has been hindered, however, by the lack of an $\alpha 2$-deficient mouse. Given that mice with null mutations in other well-studied integrins that are related to $\alpha 2\beta 1$ in either their expression pattern or function [$\alpha 1$ ($\alpha 1\beta 1$);$\alpha 3$ ($\alpha 3\beta 1$); and $\alpha 6$ ($\alpha 6\beta 1$; $\alpha 6\beta 4$)] have been available for several years and have provided considerable insight into the function of these integrins, this situation was rather surprising. Fortunately, this issue has been remedied. In this issue of *The American Journal of Pathology*, Zutter and colleagues report the generation and initial characterization of an $\alpha 2$-deficient mouse.[4] Eckes and colleagues have published similar results recently.[5]

Surprisingly, or not surprisingly, depending on your preconceived notions, mice deficient in the $\alpha 2$ subunit are quite healthy. They are viable at birth, develop normally, and are able to reproduce. Their major organs appear to be normal, at least at the level of gross histology. The overt normality of these mice, however, did not deter Zutter and colleagues from probing more deeply into their biology, using the *in vitro* studies on $\alpha 2\beta 1$ as their guide. Their observations are of considerable interest because they both confirm and refute these previously published studies, and they suggest specific areas for future work. A discussion of the phenotype of these mice and their use in future studies, however, warrants a broader discussion on collagen receptors and their relationship to $\alpha 2\beta 1$, as well as mention of the caveats associated with the interpretation of results from integrin null mice.

Collagen Receptors

Cellular interactions with and remodeling of collagen matrices, mediated by specific receptors, underlie a multitude of biological and pathobiological processes. Integrins represent one class of collagen receptors. In addition to $\alpha 2\beta 1$, four other integrins function as collagen receptors ($\alpha 1\beta 1$,$\alpha 3\beta 1$, $\alpha 10\beta 1$, and $\alpha 11\beta 1$).[6] These five integrin collagen receptors are distinguished by a common structural feature: the presence of an I (inserted) domain in their α subunits that mediates collagen binding.[6,7] Subtle differences in I domain structure among these integrins may account for the fact that these integrins differ in their ability to recognize distinct collagen subtypes.[8] For example, the $\alpha 2$ I domain binds much better to fibrillar collagens (I-III) than to collagen IV. In contrast, the $\alpha 1$ and $\alpha 10$ I domains bind to collagen IV and VI preferentially. Such differences in collagen binding specificity are relevant to the interpretation of data obtained from the analysis of mice deficient in the expression in one of the collagen-binding, integrin α subunits.

Another important feature of the integrin collagen receptors that needs to be considered with respect to the analysis of knockout mice is that they exhibit distinct patterns of tissue expression. $\alpha 1\beta 1$ is expressed primarily on cells of mesenchymal origin including fibroblasts, smooth muscle cells, and microvascular endothelial cells.[9] $\alpha 2\beta 1$ is expressed on epithelial cells, endothelial cells, fibroblasts, and cells of hematopoietic origin.[10] Interestingly, mice deficient in $\alpha 1$ expression are also viable and rather healthy, although they have defects in collagen synthesis and tumor angiogenesis.[11,12] It will be informative to generate mice deficient in the expression of both the $\alpha 1$ and $\alpha 2$ subunits given that $\alpha 1\beta 1$ and $\alpha 2\beta 1$ are co-expressed in several cell types yet interact preferentially with distinct collagens. The a3$\beta 1$ integrin is expressed in epithelia, along with $\alpha 2\beta 1$. However, the major function of $\alpha 3\beta 1$ may be as a laminin-5 receptor and not as a primary collagen receptor, although it may regulate collagen receptor function.[13] For this reason, the finding that $\alpha 3$-deficient mice die shortly after birth be-

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cause of kidney and lung dysfunction and the observation that they exhibit epidermal blistering may be attributed to the loss of laminin receptor function.[14,15] The $\alpha10\beta1$ and $\alpha11\beta1$ integrins were identified relatively recently and data on their expression and function are limited.[16–18] Mice deficient in either the $\alpha10$ or $\alpha11$ subunits have not been generated. The expression of both of these integrins, however, appears to be restricted to mesenchymal cells. $\alpha11\beta1$, in particular, is expressed on mesenchymal non-muscle cells in areas of highly organized interstitial collagen networks and it may play an important role in the organization and remodeling of these matrices during development.[18] From the perspective of $\alpha2\beta1$ function, neither $\alpha10\beta1$ nor $\alpha11\beta1$ appear to be expressed on epithelial cells or cells of hematopoietic origin.

Discoidin domain receptors (DDR1 and DDR2) are receptor tyrosine kinases that function as collagen receptors.[19] The engagement of these receptors with collagen matrices induces receptor tyrosine kinase activity, albeit with slow kinetics. Of interest, DDR1, similar to $\alpha2\beta1$, is highly expressed in epithelia, but DDR1-null mice have a more severe phenotype than do the $\alpha2$-null mice.[20] Although these mice are viable, the majority of mutant females are unable to reproduce because of improper blastocyst implantation. Those females that can reproduce are unable to lactate because of hyperproliferation and abnormal branching in the mammary glands. These data implicate DDR1 in branching morphogenesis, a function that has also been attributed to $\alpha2\beta1$.

Integrin Redundancy and Compensation in Null Mice

The subtle phenotype of the $\alpha2$-null mice raises the issues of integrin redundancy and compensation. Loss of $\alpha2\beta1$ expression in specific tissues may not be manifested in a severe phenotype because the function of $\alpha2\beta1$ may be fulfilled by other integrins with similar properties. In principle, this issue can be addressed rigorously by the generation of tissue specific knockouts in which two or more integrin subunits have been "knocked-out." The generation of an $\alpha1/\alpha2$ integrin subunit deficient mouse would exemplify this approach. A related possibility is that the loss of $\alpha2\beta1$ expression is compensated for by an increase in the expression or function of a related integrin. This possibility needs to be assessed for the $\alpha2$-null mice by careful examination of integrin expression patterns in specific tissues, especially epithelia.

Commentary on the Reported Phenotype of the $\alpha2$-Deficient Mice

Aside from gross observations, the analysis of the $\alpha2$-deficient mice to date has focused on the contribution of $\alpha2\beta1$ to platelet adhesion and hemostasis, branching morphogenesis, and epidermal wound healing.

Platelet Adhesion and Hemostasis

Numerous studies have indicated that $\alpha2\beta1$ mediates the adhesion of platelets to subendothelial collagen at sites of vessel injury and that GPVI, a low affinity co-receptor, mediates collagen-induced activation and aggregation of platelets.[2] Analysis of platelets isolated from the $\alpha2$-null mice supports this model. These $\alpha2$-deficient platelets are unable to adhere to collagen I under either static or flow conditions but they are able to aggregate in response to collagen, albeit at a decreased rate. Interestingly, platelets isolated from $\alpha2+/-$ heterozygous mice exhibit a significant reduction in their ability to adhere to collagen, a finding that is consistent with the hypothesis that the extent of platelet adhesion to collagen is a function of the level of $\alpha2\beta1$ expression. As stated,[4] the $\alpha2-/-$ and $+/-$ mice "provide an animal model to evaluate the molecular mechanisms by which allelic differences in the $\alpha2$ gene and different levels of the $\alpha2\beta1$ integrin expression are associated with increased risk of myocardial infarction, diabetic retinopathy, and stroke." Somewhat surprisingly, however, the $\alpha2$-deficient mice exhibit normal tail vein bleeding times. Zutter et al[4] suggest that other potent activators of platelets that are present at sites of vascular injury may compensate for the loss of $\alpha2\beta1$ function.

Branching Morphogenesis

The branched network of ducts in the mammary gland that is formed during puberty is a prime example of a fundamental process termed "branching morphogenesis."[21,22] Although the mechanisms that underlie this process are not well understood, it likely involves the concerted contributions of cell proliferation and matrix remodeling (both basement membrane and interstitial collagen). A key role for $\alpha2\beta1$ in this process had been indicated by the observation that this integrin is highly expressed at sites of terminal duct branching in the developing mammary gland and lung.[10] In addition, studies that mimic branching morphogenesis *in vitro* using three-dimensional collagen gels had observed that this morphogenesis is dependent on the expression and function of $\alpha2\beta1$.[23,24] Analysis of the $\alpha2-/-$ mice revealed a significant decrease in the extent of ductal branching in the $\alpha2-/-$ mice compared to the wild-type mice but branched ducts still formed. Moreover, this decrease did not hinder the ability of these mice to lactate and nurse their young. The conclusion can be drawn from these data that $\alpha2\beta1$ facilitates branching morphogenesis in the mammary gland but that it is not essential for this process. The lungs of these mice have yet to be analyzed for defects in branching morphogenesis but the fact that these mice are healthy with no overt pulmonary problems suggests that any putative lung defect is not too severe.

The availability of mammary tissue from the $\alpha2$-null mice, as well as from the DDR1-null mice, provides a wonderful opportunity to investigate the mechanism and division of labor between $\alpha2\beta1$ and DDR1 in mediating branching morphogenesis. Although the authors of both

the α2-null and DDR1-null mice studies suggest that these receptors impact branching morphogenesis by regulating epithelial cell proliferation, the process of branching is much more complex and it also involves the ability of epithelial cells to contract, degrade, and remodel extracellular matrices. Analysis of the behavior of mammary epithelial cells isolated from these null mice in three-dimensional collagen gels will be insightful and provide a foundation for more mechanistic studies aimed at assessing the contribution of these receptors to cytoskeletal dynamics, regulation of matrix metalloprotease expression, and specific signaling pathways. Moreover, the finding that the mammary glands from the α2-null and DDR1-null mice have distinct morphological phenotypes (decreased branching *versus* abnormal branching) suggests that these two receptors regulate different aspects of the branching process. Indeed, the more severe phenotype of the DDR1-null mammary glands and the inability of these mice to lactate implicate a critical role for the tyrosine kinase activity of DDR1 in mammary gland development. In this direction, targeted deletion of both the α2 subunit and DDR1 in the mammary gland would be informative.

Epidermal Wound Healing

The α2β1 integrin is expressed at relatively high levels in the basal cell layer of the skin and at moderate levels in dermal fibroblasts. Moreover, three-dimensional collagen matrices stimulate expression of the α2 subunit in dermal fibroblasts suggesting that α2β1 may be involved in the contraction of these matrices that occurs during wound healing,[25] and the migration of keratinocytes on collagen is blocked by the addition of α2-specific antibodies.[26] Collectively, these data had implicated α2β1 in the morphogenesis of the dermis and in wound healing. Studies on the α2-null mice, however, refute these predictions. The morphology of the dermis in the null mice is comparable to that of the wild-type mice, and full-thickness skin wounds in the α2-deficient mice heal as well as in the wild-type mice.

Can the *in vitro* and *in vivo* data on the role of α2β1 in epidermal wound healing be reconciled? Zutter et al[4] suggest that the α2-specific antibodies used to inhibit wound healing processes *in vitro* actually stimulate signaling pathways that impede these processes. More data are needed to support this hypothesis and the nature of the signaling pathways that impede wound healing need to be defined. Another possibility that should be considered is that loss of α2β1 function is compensated for by an increase in the expression or functional capacity of another integrin collagen receptor as discussed above. For example, both the α10β1 and α11β1 integrins are expressed on fibroblasts and could mediate collagen gel contraction. Future studies on dermal fibroblasts and keratinocytes isolated from the α2-deficient mice should prove valuable in resolving these issues.

The Role of α2β1 in Inflammation

Although the α2-deficient mice appear relatively healthy, their ability to respond to injury and infection has not been examined, with the exception of skin wounds. In fact, the α2β1 integrin was identified initially as one of several antigens (including several integrins) that are expressed during the very late stages of T cell activation (VLA-2)[27] but its specific contributions to T cell function are only beginning to be understood. The ability of α2β1 to mediate leukocyte adhesion and extravasation from the vasculature into peripheral tissues suggests that it may play an important role in inflammation. This possibility is supported by the finding that monoclonal antibodies specific for α2, as well as for α1, inhibit effector phase inflammatory responses in animal models of delayed-type hypersensitivity, contact hypersensitivity, and arthritis.[28] Also, α2β1-mediated collagen interactions may inhibit Fas ligand expression and the activation-induced cell death that occurs during the late stages of T cell activation.[29] Given that collagen I expression increases in many inflammatory and autoimmune diseases, α2β1-mediated interactions with collagen I may impede apoptosis and result in hyperactivated T cells that have the potential to contribute to tissue damage. For these reasons, studies that use the α2-deficient mice in models of chronic inflammation should provide a more rigorous assessment of the α2β1 contribution.

The Role of α2β1 in Tumor Biology

Although decreased α2β1 expression has been correlated with de-differentiation and tumor progression in the mammary gland,[3] a causal link between α2β1 and tumorigenesis has not been established. The finding that the α2-deficient mice exhibit decreased ductal branching presumably reflects a less differentiated phenotype that can be attributed to loss of α2β1 function and it suggests the value of these mice for tumorigenesis studies. In addition, as alluded to above, detailed studies on mammary epithelial cells isolated from the α2-null mice could provide important clues into the mechanism by which α2β1 contributes to epithelial differentiation.

A reasonable hypothesis based on the observations mentioned above is that the α2-deficient mice will be more prone to developing breast cancer than wild-type mice. This hypothesis can be tested by crossing the α2-null mice with one of the well-established transgenic models of mammary tumorigenesis. Mice that express erbB2, for example, require almost a year to develop breast tumors.[30] One expectation would be that α2-null mice that express erbB2 develop tumors at a faster rate.

The α2β1 integrin may also facilitate tumor progression through its contribution to angiogenesis. Senger and colleagues[31,32] have highlighted an important role for both the α2β1 and α1β1 integrins in tumor angiogenesis and have suggested that these collagen receptors function in concert with other integrins to mediate distinct components of the angiogenic process. Although no gross defects in the vasculature of the α2-null mice were noted,

the ability of tumor implanted in these mice to be vascularized needs to be examined. A similar experiment performed with the α1-null mice noted decreased vascularization with a significant reduction in capillary size and number.[12] A comparison of the α2- and α1-null mice in this regard may provide clues into the relative contribution of $\alpha 2\beta 1$ and $\alpha 1\beta 1$ to angiogenesis.

Conclusion

The generation of an α2-deficient mouse has been eagerly anticipated by the integrin community because $\alpha 2\beta 1$, a founding member of the integrin family, is one of the most-studied integrins with respect to both structure and function. The finding that the α2-deficient mice are quite healthy may be disappointing to some. Indeed, their subtle phenotype implies that many of the key functions of $\alpha 2\beta 1$ can be mimicked by other integrin or non-integrin receptors, or compensated for by an increase in the expression or function of these receptors. The analysis of these mice is far from complete, however, especially with regard to the role of $\alpha 2\beta 1$ in pathogenesis. The importance of this integrin in chronic inflammation, tumorigenesis, and angiogenesis, for example, may be substantiated or challenged by studies using these mice. Moreover, their use in conjunction with other integrin- and DDR-null mice should provide insight into the relative contribution of these receptors to specific collagen-mediated functions.

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