Vascular homeostasis: insights from a fibrotic mouse

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Efficient delivery of cancer chemotherapeutic agents depends on the permeability of the tumor vasculature. The natural hyperpermeability of tumor vessels increases absorption of macromolecular drugs into tumor tissue, suggesting that further enhancement of this process could be beneficial to the treatment of cancer (Baban and Seymour, 1998). Numerous studies have demonstrated that collagen, the most abundant protein of the extracellular matrix, plays an important role in regulating vessel permeability (Brown et al., 2003; Loeffler et al., 2006). The mechanism by which this occurs, however, had not been shown. A recent study by Sounni et al. in *Disease Models & Mechanisms* identifies a pathway by which collagen maintains vascular integrity in the Colα1(I)$^{r/r}$ mouse model (Sounni et al., 2010). In these mice, the collagenase cleavage site within type I collagen is mutated (Liu et al., 1995), causing a compensatory increase in collagenolytic activity and hemodynamic perturbation (Sounni et al., 2010). The proposed model suggests that type I collagen is a crucial component of a regulatory loop that links protease breakdown of extracellular matrix components with vascular permeability.

Sounni et al. identified matrix metalloproteinase-14 (MMP14) as the crucial protease that is activated downstream of type I collagen. This supports the earlier observation that MMPs are involved in vascular permeability (Gade et al., 2009). To determine which MMP is required for vessel stability, Sounni et al. conducted a series of in vivo assays to assess vascular leakage in MMP-knockout mice (see figure 1B in Sounni et al., 2010). The enhanced steady-state leakage in *MMP14*-null mice implicated MMP14 as the metalloproteinase that is crucial for maintaining vascular stability in this model (see figure 1D in Sounni et al., 2010). In addition, Sounni et al. employed the Colα1(I)$^{r/r}$ mouse model to demonstrate the influence of increased collagen levels on MMP activity and vessel stability. As expected, the Colα1(I)$^{r/r}$ mouse displayed increased MMP activity and decreased vascular permeability (see figure 2A,B in Sounni et al., 2010). The broad-spectrum metalloproteinase inhibitor GM6001 rescued this phenotype. Although the authors interrogated the influence of the related metalloproteinase MMP2 on their proposed mechanism, the potential off-target effects of GM6001 on other MMPs should also be considered. Use of MMP14-specific antibodies, which have recently become available (Devy et al., 2009), would serve to strengthen these data. However, the results strongly suggest that the overabundance of uncleaved collagen in the Colα1(I)$^{r/r}$ mouse induces hyperactivation of MMP14, which in turn decreases vascular permeability.

The observation that MMP14 can activate latent transforming growth factor-β (TGFβ) (Mu et al., 2002; Werb, 1997) led Sounni et al. to hypothesize that TGFβ might be the downstream mediator of vascular permeability. The authors supported this assertion through additional work in the Colα1(I)$^{r/r}$ mouse. TGFβ levels were found to be elevated in the Colα1(I)$^{r/r}$ mouse when compared with the wild-type control (see figure 4B in Sounni et al., 2010). In addition, MMP14-knockout mice showed reduced levels of TGFβ. The authors validated the direct effect of collagen and MMP14 on TGFβ bioavailability through in vitro studies in a cell line overexpressing MMP14 and grown on collagen isolated from Colα1(I)$^{r/r}$ mice (see figure 4H in Sounni et al., 2010). These cell lines exhibited increased TGFβ activity, as measured by a luciferase reporter assay. To demonstrate that TGFβ directly mediates vascular permeability, Sounni et al. injected TGFβ-neutralizing antibodies into the Colα1(I)$^{r/r}$ mice before challenging them with mustard oil, and observed rescue of vascular permeability (see figure 4I in Sounni et al., 2010). Inhibition of the TGFβ receptor, ALK5, also restored the vascular response in these mice. As a final verification that this phenomenon was not restricted to Colα1(I)$^{r/r}$ mice, the authors used an inducible TGFβ bigenic mouse model. As predicted, overexpression of TGFβ resulted in decreased vessel permeability (see figure 5B in Sounni et al., 2010). Taken together, these data strongly support a mechanism of collagen-induced MMP14 activation, leading to the release of active TGFβ and a subsequent decrease in vascular permeability.

Finally, Sounni et al. showed that inhibition of this pathway results in increased uptake of high-molecular-weight compounds into tumor tissue. TGFβ blockade with an ALK5 inhibitor increased dextran delivery to tumor tissue in an MMTV-PyMT mouse model of breast cancer (see figure 6E in Sounni et al., 2010). Although suggestive, the ability of TGFβ blockade to enhance the effectiveness of chemotherapy was not assessed. Furthermore, this study did not consider that TGFβ acts as a tumor suppressor early in neoplastic growth and promotes tumor progression later in the disease (Pardali and ten Dijke, 2009). Therefore, inhibiting TGFβ early in primary or secondary tumor development might actually promote tumor growth. Last, the influence of TGFβ seems to be dependent upon the cellular microenvironment. In contrast to the stabilizing effect of TGFβ on the vasculature observed by Sounni et al., TGFβ has also been shown to increase the permeability of endothelial cells in culture (Birukova et al., 2005). These data suggest that TGFβ inhibition elicits differential responses depending on both tumor stage and tumor microenvironment. Careful consideration must therefore be taken when extrapolating the potential advantages of manipulating the pathway described by Sounni et al. (Sounni et al., 2010).

Regardless of the therapeutic implications, the data presented by Sounni et al.

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Table 1. Summary of results presented by Sounni et al., 2010

Figure	Mouse	Inhibitor target	Vascular leakage
1A	Wild type	MP	+
1C	*MMP14*$^{-/-}$	–	+
2C	Colα1(I)$^{r/r}$	–	–
2C	Colα1(I)$^{r/r}$	MP	+
4I	Colα1(I)$^{r/r}$	TGFβ	+
4J	Colα1(I)$^{r/r}$	ALK5	+
5B	TGFβ ind	–	–

Vascular leakage compared with controls, as assayed by leakage of Evans Blue dye into interstitial tissue from the ears of control and drug-treated or mutant mice (Sounni et al., 2010). MP, metalloproteinase; TFGβ ind, TFGβ-inducible.

(summarized in Table 1) uniquely address the role of the collagen-MMP14-TGFβ pathway in vascular homeostasis, as demonstrated through use of the Colα1(I)$^{r/r}$ mouse. Their work provides mechanistic evidence for the role of TGFβ in stabilizing vessels after acute insult, and for the role of type I collagen in regulating vascular permeability (see figure 7 in Sounni et al., 2010). In conclusion, these data provide a platform for further investigation and a rationale for testing the effects of manipulating vascular permeability during chemotherapeutic intervention.

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