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A pathophysiological role for heparin-binding EGF-like growth factor in myocardial injury

The biological function of tissue trophic factors is of obvious importance in development. What has become overwhelmingly clear is that secreted factors have enormous influence on the ability of tissues to recover from injury. A case in point is the recovery of the myocardium from infarction. Insulin-like growth factor (IGF), hepatocyte growth factor (HGF), and heparin-binding EGF-like growth factor (HB-EGF) all have emerged as cardiogenic and cardi hypertrophic growth factors. For instance, HB-EGF-null mice develop severe heart failure after birth. HB-EGF is synthesized as a type-I transmembrane protein (proHB-EGF); this form of the protein may be mitogenic or growth inhibitory, depending upon the cell type. Membrane-bound proHB-EGF is cleaved by a specific metalloproteinase, releasing soluble HB-EGF. The latter is a potent mitogen for vascular smooth muscle cells, fibroblasts, and other cell types. While the beneficial effects of IGF and HGF have been well demonstrated in injured hearts, it is unclear whether the natural upregulation of HB-EGF expression that occurs following cardiac injury benefits or impairs the recovery of the heart. In this issue, **Ushikoshi et al**¹ (p. 862) utilized adenoviral HB-EGF gene transduction *in vitro* and *in vivo* to demonstrate that overexpression of HB-EGF had no cytoprotective effect on Fas-induced apoptosis or oxidative stress injury in primary cultured mouse cardiomyocytes. Absent injury, HB-EGF was capable of inducing hypertrophy of cardiomyocytes and proliferation of cardiac fibroblasts. *In vivo*, local overexpression of HB-EGF in the border areas of a myocardial infarction in rabbits did not improve cardiac function or promote angiogenesis. These studies were performed utilizing an adenoviral vector, which gave greater temporal control of HB-EGF expression than would be possible with a murine transgenic overexpression approach. In this rabbit model, HB-EGF increased levels of apoptosis and fibrosis in the infarcted region following reperfusion, and increased the numbers of myofibroblasts and macrophages. Myocardial hypertrophy only occurred elsewhere in the left ventricle. Hence, overexpressed HB-EGF in the infarcted region did not result in a beneficial outcome, but rather exacerbated the remodeling process through the mitogenic activation of fibroblasts into myofibroblasts, deposition of fibrous tissue, and the recruitment of

inflammatory cells. Moreover, HB-EGF was not cytoprotective, as it did not inhibit apoptosis of cardiomyocytes under conditions of oxidative stress as would occur in the postinfarction state. Myocardial remodeling following myocardial infarction may therefore be guided down a pathogenic pathway of fibrosis and aberrant remodeling as a result of HB-EGF expression. This novel observation has significant bearing in the understanding and potential therapy of heart injury, including recovery from myocardial infarction.

Reference

- 1 Ushikoshi H, Takahashi T, Chen X, *et al*. Local overexpression of HB-EGF exacerbates remodeling following myocardial infarction by activating non-cardiomyocytes. *Lab Invest* 2005;85:862–873.

Progress in understanding colitis: a role for epithelial CD98

CD98 is a cell surface glycoprotein composed of covalently linked heavy and light chains. It was originally described as a lymphocyte activation antigen, but in polarized epithelia CD98 is localized to the basolateral domain where it functions as an amino-acid transporter and regulator of β 1 integrin function. Epithelial CD98 can also interact with epithelial intercellular adhesion molecule I (ICAM-1). Together with a variety of functional data these observations raise the possibility that adhesion and amino-acid transport activity may be coordinately regulated by interactions between CD98, ICAM-1, and β 1 integrins. In this issue, **Kucharzik et al**¹ (p. 932) extend previous reports of increased CD98 expression on lymphocytes and epithelia in response to TH1 cytokines. Since epithelial CD98 regulates β 1 integrins, which may be involved in inflammatory disease, the authors hypothesized that epithelial CD98 might also be involved in intestinal disease. They first showed that epithelial CD98 was upregulated in intestinal epithelial cells exposed to interferon- γ *in vitro*. They then demonstrated similar upregulation *in vivo* following DSS exposure, a standard murine model of colitis. This increased CD98 expression did not occur in interferon- γ knockout mice, demonstrating the critical *in vivo* role of interferon- γ in regulation of CD98 expression. Further studies showed that administration of an activating anti-CD98 monoclonal antibody worsened colitis in DSS-treated mice, demonstrating a functional role for CD98 in colitis. To determine if the effect of the anti-CD98 monoclonal antibody could be due to targeting of epithelial cells, *in vitro*

studies were performed using cultured intestinal epithelial cells. These showed that anti-CD98 monoclonal antibody slowed wound closure in intestinal epithelial cell monolayers. Therefore, activation of epithelial CD98 plays an important role in progression, or perpetuation, of intestinal inflammation. This observation may lead the way for future studies of anti-CD98 blocking antibodies as potential therapeutic agents.

Reference

- 1 Kucharzik T, Luger A, Yan Y, *et al.* Activation of CD98 glycoprotein perpetuates colonic inflammation. *Lab Invest* 2005;85:932–941.

The FLIP side of tumor cell apoptosis

The ability to target and selectively kill tumor cells without damage to normal tissue represents a 'Holy Grail' of human cancer therapy. Members of the tumor necrosis factor (TNF) ligand superfamily include Fas/FasL and Apo2L/TRAIL; their respective receptors have attracted much attention over the past decade as selective tumor executioners. Perhaps the most intensely studied has been TRAIL (TNF-related apoptosis-inducing ligand, also called Apo2L), which binds to two functional death receptors (DR4, DR5) and three nonfunctional 'decoy' receptors (DcR1, DcR2, and osteoprotegerin). Apo2L/TRAIL has been shown to induce apoptosis in many tumor types via a complex intracellular pathway involving recruitment of the Fas-associated death domain protein (FADD), which ultimately leads to activation of caspase-8 and caspase-3. However, TRAIL-induced cell death is negatively regulated by several polypeptides including the cellular FADD-like interleukin 1-converting enzyme (FLICE)-inhibitory protein (FLIP). FLIP shares a high degree of homology with caspase-8 but lacks proteolytic activity. Inhibition of caspase-8 activity by increased expression of FLIP would give tumor cells a survival advantage, making them less susceptible to therapeutic efforts using Apo2L/TRAIL or related agonists.

In this issue of *Lab Invest*, Dolcet *et al*¹ (p. 885) studied human endometrial carcinoma to provide evidence that FLIP inhibits apoptosis triggered by death receptors. First, a tissue microarray containing surgical specimens of 96 endometrial carcinomas revealed FLIP immunoreactivity in about half of cases and a positive correlation between FLIP reactivity and tumor stage. Next, in a series of experiments using two endometrial carcinoma cell lines, the authors show that TRAIL-induced apoptosis is inhibited by overexpression of FLIP (using transient transfection) while downregulation of endogenous FLIP by siRNA transfection resulted in increased caspase-8 activation and restoration TRAIL-induced killing. Also inside this issue, Jeon *et al*² (p. 874) report studies that implicate

FLIP as a negative regulator of Fas-mediated apoptosis in EBV-associated sinonasal NK/T-cell lymphoma. The authors found that EBV-positive NK/T lymphoma cell lines were resistant to Fas-mediated apoptosis induced by anti-Fas antibodies, despite having high surface expression of nonmutated Fas. Treatment of cells with cyclohexamide (a protein synthesis inhibitor) enhanced Fas-mediated apoptosis and resulted in marked reduction of FLIP while other anti- and proapoptotic members of the bcl-2 family were not significantly altered. *In vitro* findings were supported by immunohistochemical studies showing that FLIP was expressed in about 40% of NK/T-cell lymphomas resected from patients with the disease.

These two studies support a basic mechanism by which upregulation of FLIP promotes improved survival and chemoresistance in two distinctly different forms of neoplasia. It follows that researchers developing targeted antiapoptotic cancer therapies should consider the 'FLIP side' in future laboratory investigations.

References

- 1 Dolcet X, Llobet D, Pallares J, *et al.* FLIP is frequently expressed in endometrial carcinoma and has a role in resistance to TRAIL-induced apoptosis. *Lab Invest* 2005;85:885–894.
- 2 Jeon YK, Kim H, Park SO, *et al.* Resistance to Fas-mediated apoptosis is restored by cyclohexamide through the downregulation of FLIP_L in NK/T-cell lymphoma. *Lab Invest* 2005;85:874–884.

Malignant transformation of thymoma in the setting of HTLV-I

The human T lymphocyte virus type-I (HTLV-I) is causally implicated in adult T-cell leukemia/lymphoma and is associated with several autoimmune and inflammatory disorders. Tax, encoded by the pX gene of HTLV-I, is a protein that modulates the function of growth factors, transcription factors and cellcycle-related molecules such as p53 and p16ink4a that are crucial for cell survival and proliferation. For this reason, the pX gene is thought to play a major role in HTLV-1 tumorigenesis.

Very few animal models are available to study tumorigenesis in thymomas. Kikuchi *et al*¹ reported earlier that transgenic rats carrying the pX gene under the control of a rat lymphocyte-specific protein tyrosine kinase type-I promoter (lck-pX rats) often develop epithelial thymomas that are considered benign since they never invade neighboring organs or metastasize. In this issue, the same group (Tsuiji *et al*,² p. 851) describes the malignant transformation of epithelial thymomas induced by heterotopic transplantation of thymuses from newborn lck-pX rats into the subcapsular space of kidneys of thymectomized lck-pX rats. At 3 months after transplantation, tumors histologically similar

to the benign thymomas were present in the subcapsular space, indicating that thymomas can also develop at heterotopic sites. At 6 months, however, malignant transformation occurred, attributed to the presence of local tissue factors. In comparison to the benign thymomas, the malignant tumors were highly proliferative, metastatic, and transplantable. In addition, the expression of the pX transgene in the malignant thymomas was significantly reduced, and the expression of critical host genes such as the cyclin-dependent kinase inhibitor p16ink4a/ARF, which was significantly upregulated in the benign thymomas, was undetectable in the malignant forms, most likely due to a homozygous gene deletion. It is therefore worth noting that p16ink4a and ARF are tumor suppressor genes, and abnormalities of these genes are present in a significant number of human malignancies. The findings of Tsuji *et al* suggest that p16ink4a expression may play a role in thymoma transformation. In benign thymomas with aberrant expressions of the pX gene, the high levels of p16ink4a expression may

control tumor growth. Deletion of this gene and alteration of cell cycle regulation may lead to a state of higher malignancy. Furthermore, the results suggest that the transformed tumor cells may not require the expression of pX gene for further growth. The exact process of thymoma transformation in this model system remains unclear. Regardless of the mechanism involved, however, this rat model may become a very useful tool in the study of tumorigenesis of thymomas *in vivo*.

References

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- 2 Tsuji T, Ikeda H, Tsuchikawa T, *et al*. Malignant transformation of thymoma in recipient rats by heterotopic thymus transplantation from HTLV-I transgenic rats. *Lab Invest* 2005;85:851–861.