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EGFR amplification and overexpression in breast phyllodes tumors

Originally called 'cystosarcoma phylloides' by Muller in 1838, phyllodes tumors (also spelled 'phylloides') account for about 1% of breast neoplasia. Such tumors occur primarily in adults, with a median age at diagnosis of 45 years. They are composed of epithelial elements that line frond or leaf-like masses of stromal cells. Benign tumors rarely metastasize but may locally recur. Malignant neoplasms carry a 3–12% risk of metastasis. Malignancy in breast phyllodes tumors is defined by changes in the stromal element including marked nuclear atypia, increased mitoses, and overgrowth of the epithelium. Owing to its rarity and lack of experimental models, little is known about the molecular mechanisms of tumorigenesis and progression of this tumor.

In this issue, **Kersting *et al***[1] (p. 54) used a tissue microarray to analyze a series of 58 breast phyllodes tumors for expression levels of epidermal growth factor receptor (EGFR) and studied *egfr* gene amplification including amplification of a short CA repeat within intron 1. Positive immmuoreactivity for EGFR was detected in 19% of all tumors studied but was present in 75% of the malignant phyllodes tumors. There was a significant correlation between EGFR overexpression and intron 1 gene amplification by FISH. However, neither EGFR overexpression nor whole gene amplification were found in a series of 167 fibroadenomas that were also studied. These findings suggest a role for epidermal growth factor signaling in the stromal element of breast phyllodes tumors and may spark further investigations into the pathobiology of these enigmatic tumors.

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Beneficial effect of G-CSF on established heart failure after myocardial infarction

Postmyocardial infarction (MI) heart failure is a major complication for survivors of acute MI. Recent studies using experimental animals have demonstrated that a hematopoietic cytokine, granulocyte colony-stimulating factor (G-CSF) can improve cardiac function and reduce mortality following MI. However, in these previous studies, administration of G-CSF was started before or within days after the induction of acute MI. Therefore, it remains unknown whether G-CSF can ameliorate established heart failure, in the weeks after an acute MI.

In a study published in this issue of *Lab Invest*, **Li *et al***[1] (p. 32) administered G-CSF 12 weeks after mice were subjected to acute MI. The G-CSF treatment improved the function of murine hearts, inducing hypertrophy among surviving cardiomyocytes and reducing myocardial fibrosis. The observed effects of G-CSF on cardiac function lasted at least for 2 weeks after discontinuation of the treatment.

The mechanisms by which G-CSF ameliorates post-MI cardiac failure are controversial. The present study demonstrated elevated expression of G-CSF receptor in failing hearts. Moreover, G-CSF treatment induced activation of Stat3 and Akt, followed by an upregulation in signals involved in cardiomyocyte hypertrophy and antifibrosis such as GATA-4, MHC, MMP-2 and MMP-9. These data are largely consistent with observation in a recent publication using G-CSF at the acute phase of MI.[2] In contrast, antiapoptotic effects were not likely involved in this chronic heart failure model, because apoptosis was already rare. Furthermore, no bone marrow-derived cardiomyocytes or vascular cells were detected in the failing hearts using chimeric mice transplanted with GFP bone marrow cells.

Overall, the study by Li *et al* implicates a potential benefit of G-CSF in patients suffering from chronic heart failure after MI, and provides us with mechanistic insights both supporting and contradicting previous studies.

References

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Totally tubular?

Acute tubular necrosis (ATN), caused by ischemia or other nephrotoxic insults, is the most common cause of acute renal failure in hospitalized patients. The renal failure that accompanies ATN is char-

acterized histologically by tubular epithelial cell necrosis and apoptosis. Subsequent tubule repopulation restores normal tubular architecture and function. Treatments that augment this repair process could shorten hospital stays and reduce the need for dialysis. The identity of the cell population responsible for the repair of the tubules is, however, unknown. Do renal tubules, like many epithelial structures, have resident, pluripotent, lineage restricted stem cells that can be mobilized in response to damage? Or are bone marrow-derived cells, which have been shown not to be restricted to a hematopoietic lineage, involved?

A variety of evidence in both humans and mice support the ability of bone marrow-derived cells to become renal tubular cells. These studies are based on transplantation of bone marrow-derived cells (BMCs) that differ phenotypically or genetically from the host. The most commonly used genetic markers are the Y-chromosome or transgenes, such as GFP or β-galactosidase. For example, tubule cells containing a Y-chromosome can be detected in kidneys of male humans, and mice, who have received kidneys from a female donor. More interestingly, infusion of bone marrow-derived mesenchymal stem cells into mice limited loss of renal function and tubular damage induced by cisplatin, suggesting that these cells are involved in repair and represent a potential treatment for ATN.[1] However, the frequency with which BMCs are reported to contribute to tissue repair in the kidney and other epithelial organs varies widely.

In this issue of *Lab Invest*, **Toyokawa *et al***[2] (p. 72) use sophisticated imaging techniques to assess the contribution of BMCs to renal tubular repair. They demonstrate that two-dimensional imaging is simply insufficient to properly evaluate the contribution of hemopoietic cells to tissue repair. Toyokawa *et al* show that two-dimensional imaging overestimates the frequency of BMCs within renal tubules during repair. This careful comparison of the two-dimensional methods used previously with an elegant three-dimensional approach may explain the contradiction between early studies which concluded that BMCs contributed significantly to tubular repair and more recent studies concluding that resident kidney cells are the principal source of new epithelial cells, with BMCs making, at most, a minor contribution.[3] Toyokawa *et al* used transgenic rats expressing enhanced green fluorescent protein (EGFP) as BMC donors for transplantation into wild-type EGFP-rats and, conversely, transplanted wild type EGFP-BMCs into EGFP-transgenic rats. At 6 weeks after BMC infusion rats were subjected to renal ischemia reperfusion injury using standard models. The presence, or absence, of EGFP, depending on the BMC source, was used to mark donor-derived cells and a simple F-actin stain helped to identify tubular epithelial cells. Thus, identification of BMCs within tubules depended upon the presence of EGFP within a cell possessing an epithelial F-actin staining pattern. Standard two-dimensional imaging immunofluorescent imaging of 6 μm sections suggested that between 0.18 and 0.68% of cells in the reconstituted tubules were of BMC origin. The rate was similar, regardless of whether EGFP+ or EGFP− cells were transferred, suggesting that EGFP transgene silencing is not responsible for the low frequency of tubular BMCs observed. Of course, the interstitium of the kidney is full of cells of hematopoietic origin that have developed along a hematopoietic lineage and would also express the GFP transgene. Could what looks like colocalization of GFP and F-actin in reality be two closely opposed cells, each staining with a single marker? In a parallel set of experiments the authors used the same experimental conditions and then took 50 μm sections. Confocal optical sections were collected at 0.3 μm intervals and a three-dimensional image was constructed. These three-dimensional data demonstrate that as few as 0.1% of the cells in the reconstituted tubules are of BMC origin. The authors therefore conclude that up to three-fourths of the cells identified as of BMC origin in two-dimensional imaging represent false positives. Thus, the major contribution to renal tubular repair comes from cells other than the BMCs. This work clearly demonstrates the difficulty in assessing three-dimensional structure from two-dimensional sections and confirms the importance of using three-dimensional techniques in assessing the involvement of BMCs in epithelial repair. Indeed, a similar three-dimensional analysis of colonic epithelium has recently been used to demonstrate the role of BMCs in colonic epithelial repair.[4] Thus, it appears that the ability of BMCs to limit renal injury may reflect a role independent of transdifferentiation,[5] leading us to conclude that the contribution of BMCs to renal repair is not totally tubular.

References

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Pushing adult liver towards pancreatic differentiation

In the midst of the vigorous discussion of whether bone marrow stem cells can cross the mesodermal barrier to reconstitute endodermal tissues (see 'Totally Tubular?' above[1]), the question also can be asked whether more proximate progenitor cells can be used for regenerative purposes. The embryology of the liver and pancreas provides a particular opportunity, as these organs arise out of a common endodermal precursor epithelial layer. The 'master control gene' Pancreatic-duodenum homeobox protein-1 (Pdx1) is particularly critical for initiating the pancreatic differentiation pathway in this embryologic cell layer.

When adult hepatic progenitor cells are transduced with Pdx1, pancreatic differentiation also can be initiated. This finding has engendered intense interest in whether adult hepatic progenitor, or 'stem' cells, can be used for the treatment of type 1 diabetes. Unfortunately, both exocrine and endocrine programming ensue when hepatic stem cells are transduced with Pdx1. Indeed, a robust hepatitis can develop if these transduced cells reside in the liver *in vivo*. Yang and colleagues have previously demonstrated that progenitor cells derived from the adult rat liver can be reprogrammed *in vitro* towards pancreatic endocrine insulin-producing cells. This is achieved through transduction with Pdx1-VP16, a fusion protein of Pdx1 and the activating domain of the viral VP16 transcription factor, and subsequent culture in a high glucose (20 mM) media.[2] Such cells were capable of ameliorating hyperglycemia in the spontaneously diabetic NOD-scid mouse. In the current issue, **Tang *et al***[3] (p. 83) tackled the issue of whether transdifferentiation strategies could be improved, in particular to examine whether vector efficiency played a role in obtaining an successful endocrine outcome. A comparison was made between *in vitro* transduction of rat hepatic stem-like WB cells with either Pdx1 or Pdx1-VP16. Unlike the previous study in which adenovirus was used for short-term transduction, lentivirus was used in the current study and outcomes followed short term (6 days) and long term (up to 3 months). After lentivirus transfection with either Pdx1 or Pdx1-VP16, single cell WB clones were derived and cultured under standard conditions (in the absence of high glucose concentrations), or in the presence of 20 mM glucose. In the short term, lentivirus-Pdx1-VP16 was found to be more efficient in initiating pancreatic endocrine differentiation under high glucose conditions, when compared to lentivirus-Pdx1 alone. In the long term, the two transduction strategies were equally effective in generating sustained pancreatic insulin-producing cells. Both Pdx1- and Pdx1-VP16-expressing WB cells were able to reverse diabetes following renal subcapsular implantation into diabetic NOD-scid mice. Both sets of implanted cells showed a similar ability to respond to a glucose challenge, as indicated by an intraperitoneal glucose tolerance test. Such findings demonstrate that liver-derived progenitor cells can be induced to transdifferentiate into insulin-producing cells *in vitro*, and can become fully functional when exposed to an *in vivo* diabetic microenvironment. There was no dramatic difference between the effects of long-term expression of Pdx1 and Pdx1-VP16, with regards to pancreatic gene expression *in vitro*, or the capacity to become functional insulin-producing cells in the diabetic microenvironment *in vivo*. The current study thus shows that long-term transduction of hepatic stem cells with lentivirus vectors provides more consistent pancreatic endocrine transdifferentiation, than the short-term induction gained using adenovirus vectors. Moreover, while the Pdx1-VP16 fusion protein shows advantage over native Pdx1 in the immediate short-term following lentiviral transduction, both proteins during long-term culture of liver stem cells *in vitro*. Most importantly, diabetes can be effectively reversed in diabetic mice, following implantation of these transdifferentiated cells into the renal subcapsule. This paper therefore provides insight into how stem cell manipulation can be optimized, and pushes the possibility of stem cell therapy for diabetes just that much closer to reality.

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