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Overexpression of a secreted protein in acrania: whoa NELL(IE)-1!

Congenital or acquired malformations of the skull are rare but important disease entities that involve loss of this structure (acrania, acalveria) or disordered cranial fusion (craniosynostosis). Acrania is the generic term for partial or total absence of the skull. It may occur with or without normal brain development. For example, anencephaly is a rather common neural tube defect in which no skull is formed secondary to loss of critical inducing elements provided by the developing brain. In contrast, acalvaria is a rare malformation consisting of absence of the calvarial bones, dura mater and associated muscles in the presence of a normal skull base with the central nervous system being typically unaffected. The pathogenesis of developmental skull defects is believed to be multifactorial and the molecular disease mechanisms remain poorly understood.

In this issue, **Zhang *et al*¹** (p. 633) present a transgenic mouse model in which acrania-like defects are induced to occur by overexpression of NELL-1. Prior work from the author's laboratory had identified *NELL-1* as an overexpressed gene in patients with unilateral craniosynostosis. The gene was subsequently found to encode a secretory protein with an NH2-terminal thrombospondin (TSP)-like module, five von Willebrand factor C domains, and six EGF-like domains. NELL-1 is normally expressed during fusion of the coronal sutures in neural crest derived tissues including intramembranous cranial bone and neural tissue. This group also reported a *Nell-1* transgenic mouse that developed various craniosynostosis phenotypes.

In the present study, the authors report that acrania-like cranioskeletal malformations occur among *NELL-1* transgenic mice. Several mice had encephaloceles characterized by occipital bone defects with brain herniation. There was increased apoptosis of calvarial bone osteoblasts and premature hypertrophy and apoptosis of chondrocytes throughout the distorted chondrocranium of *NELL-1* transgenic mice, compared to wild-type littermates. The observed apoptosis was associated with an increase in Fas and Fas-L production. These results suggest that acrania-like developmental defects could involve abnormal expression of *NELL-1* leading to aberrant apoptosis by altering Fas-related cell death pathways.

Reference

- 1 Zhang X, Cowan CM, Jiang X *et al*. *Nell-1* Induces acrania-like cranioskeletal deformities during mouse embryonic development. *Lab Invest* 2006;86:633–644.

Learning about cancer from embryonic lethal mice

Among the great breakthroughs in medical science in the last century, elucidation of the molecular genetics of cancer syndromes ranks high. After identification of candidate gene mutations in the human, a valuable experimental paradigm is to explore the function of the gene in animal models. Abrogation of function through homozygous deletion of the murine gene homolog is particularly revealing. Unfortunately, when homozygous deletion creates an embryological lethal outcome, the mouse experiment ends all too quickly. Such is the case for examination of the gene whose mutation underlies von Hippel-Lindau (VHL) disease. VHL is a hereditary cancer syndrome, which is transmitted in an autosomal dominant manner and affects approximately 1 in 36 000 individuals. In the human, *VHL* germline mutations give rise to well-vascularized tumors, including hemangioblastomas of the retina and central nervous system, pheochromocytomas, and renal cell carcinomas. The *VHL* gene maps to 3p25; the protein is a component of an E3 ubiquitin ligase complex that recognizes and degrades the alpha subunits of hypoxia-inducible factor (HIF) under normoxic conditions. HIF in turn is a transcription factor that regulates cellular responses to changes in oxygen concentration, by regulating the expression of genes involved in angiogenesis, hematopoiesis, pH regulation and energy metabolism. These target genes play a critical role in tumor growth and progression. In murine studies of the *VHL* gene, heterozygous *VHL*-knock-out mice develop vascular hepatic tumors. However, the homozygous knockout mouse has an early embryonic lethal outcome.

To overcome the early embryonic lethality of the *VHL*-null mouse, **Hong *et al*¹** (p. 664) introduced a tamoxifen-inducible *Cre* (*CreER*TM) transgene, enabling time-specific abrogation of the *VHL* gene at specific points during embryologic development. Acute tamoxifen-induced inactivation of the *VHL* gene at E10.5 resulted in embryonic lethality between E14.5 and 15.0, associated with severe hemorrhage and abnormal vasculature in the liver, and abnormal circulation in the yolk sac. In fibroblasts *in vitro*, inactivation of *VHL* led to induction of the HIF target genes vascular endothe-

lial growth Factor (VEGF) and carbonic anhydrase IX (CAIX). By isolating *VHL* inactivation to a specific embryonic stage, these authors thus demonstrated that the *VHL* gene plays a key role in vascular development during embryogenesis. This system may thus provide valuable opportunity for *in vivo* study of genes that cause early embryonic lethality. Perhaps more importantly, there is interest in inhibition of angiogenesis and HIF-regulated genes as a potential treatment for cancer. These animals may be a valuable system for *in vivo* testing pharmacologic inhibitors of VEGF or HIF, with survival of *in utero* development as the experimental readout.

Reference

- 1 Hong SB, Furihata M, Baba M, *et al.* Vascular defects and liver damage by the acute inactivation of the *VHL* gene during mouse embryogenesis. *Lab Invest* 2006;86:664–675.

Derivation of premeiotic male germ cells from adult bone marrow?

The unexpected plasticity of bone marrow cells has been one of the most controversial issues in current medical sciences. As this issue is of both concern and interest to the Pathology community, *Lab Invest* has been dealing with the subject occasionally, in particular by publishing provocative original manuscripts or outstanding reviews.^{1,2}

In a study published in this issue, **Nayernia *et al.***³ (p. 654) describes the isolation and characterization of bone marrow cells with the potential to exhibit properties of spermatogonial stem cells. They used *Stra8*-GFP transgenic mice that express GFP specifically in male germ cells. Of interest, GFP-positive cells were isolated from bone marrow cultures treated with retinoic acid (RA). The GFP-expressing cells in marrow were found to also express germ cell markers such as *Mvh*, *Oct4*, *Dazl*, *Piwil2*, etc. RA is apparently necessary for germ cell marker expression. To test for developmental competence, the cells were transplanted into testes of mice previously depleted of spermatogonial stem cells using busulfan treatment. While the cells appeared to be able to colonize into the seminiferous epithelium, they were apparently incapable of further meiotic differentiation.

Recently, Jonathan Tilly's group proposed continuous immigration of germline stem cells derived from adult bone marrow into mouse ovaries,⁴ and generated an immense controversy.⁵ Not the least concern is the premise that 'safe' stem cell therapy should not influence the germ cell lineages of a recipient. The current paper by Nayernia *et al.* adds further weight to the issue. Evidently, the 'marrow to germ cells' phenomenon is the newest and perhaps the biggest challenge to stem cell therapy and regenerative medicine. The implications for normal

mammalian developmental biology also are considerable, since these findings challenge the premise that germ cell lines are established *in utero* and remain inviolate thereafter.

References

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- 2 Jung Y, Oh S-H1, Zheng D, *et al.* A potential role of somatostatin and its receptor *SSTR4* in the migration of hepatic oval cells. *Lab Invest* 2006;86:477–489.
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- 5 Telfer EE, Gosden RG, Byskov AG, *et al.* On regenerating the ovary and generating controversy. *Cell* 2005;122:821–822.

Exploration down the FOXP3 pathway identifies two key target genes for T_{reg} programming

The last few years have witnessed the amazing revival of regulatory T cells, once scorned by most immunologists. Now most of the skeptics have been convinced of the existence of a T-cell-mediated form of dominant tolerance. Defects in regulatory T cells have now been associated with many autoimmune diseases (type 1 diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, to cite the most prevalent ones) in both patients and corresponding animal models. Their role has also been invoked in the ability of certain tumor cells or pathogens to evade immune surveillance. The field is complex, as several T-cell subsets are able to inhibit the effector functions of other T cells, and the mechanisms by which this inhibition occurs are still the subject of intense debate. The most studied subset of regulatory T cells is undoubtedly the CD4⁺ T cells that express high levels of the IL-2 receptor α chain CD25 (CD4⁺ CD25⁺ or T_{reg}). A significant advance in the understanding of T_{reg} biology has been achieved by the identification of a master regulator of this lineage, FOXP3. FOXP3 is a transcription factor from the forkhead family, and null mutations in *Foxp3* have been identified as the cause of a severe multiorgan autoimmune disease in immunodysregulation, polyendocrinopathy, enteropathy, X linked syndrome (IPEX) patients and in the *scurfy* mouse. Genetic manipulations have shown that, at least in mice, FOXP3 expression is largely restricted to regulatory CD4⁺ T cells, and that the expression of this transcription factor was necessary and sufficient for the T_{reg} phenotype. However, the more promiscuous expression of human FOXP3

limits to a certain extent the targeting of this molecule for therapeutic purposes. Although a number of FOXP3 terminal gene targets have been identified by gene array, more proximal targets play a specific role in establishing and maintaining the regulatory phenotype remain elusive.

In this issue, **Ocklenburg *et al***¹ (p. 724) have used a lentiviral vector to overexpress FOXP3 in human CD4⁺ T cells and showed that, contrary to the mouse, it induced only a partial regulatory phenotype as compared to naturally occurring T_{reg} cells. Using microarrays, these investigators identified a number of differentially expressed genes in both naturally occurring T_{reg} and in FOXP3-transduced T cells. Two of these genes are then analyzed in detail: ubiquitin-like gene diubiquitin (UBD) and the β -galactoside binding lectin LGALS3. They found that UBD is a key player in the regulation of anergy in T_{reg}, downstream of FOXP3. Ubiquitin ligases have been previously associated to anergy induction; the novelty of the results presented here is to associate UBD, whose expression in T cells was not known, to T_{reg} anergy induction. LGALS3 is expressed at high levels in T_{reg} and in FOXP3-transduced T cells, but not in UBD-transduced T cells, indicating that these two targets are expressed independently from each

other. LGALS3 appears to be a T_{reg}-specific signature of antigen-stimulated human CD4⁺ CD25-derived T_{reg} cells. LGALS3 has been previously described to induce T-cell apoptosis. Blocking LGALS3 did not impair T_{reg} suppression function, however, leaving the function of this signature molecule yet to be elucidated. Interestingly, murine LGALS3 is broadly expressed, indicating that murine models will not be useful to unravel the function of this molecule in human T_{reg} cells.

This work represents an important step in the understanding of the molecular machinery that programs a T cell with the ability of active immunosuppression. A detailed understanding of this machinery is necessary to master therapeutic manipulations of this cell compartment, in which many are holding high hopes for successful regulation of immunologically mediated diseases.

Reference

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