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Nestin gene expression and regulation in proliferating endothelium: 'V-nestin'

Intermediate filaments (IFs) are cytoskeletal proteins that form 8–10 nm fibrils, which along with microfilaments (7 mm) and microtubules (24 mm), help define the shape and polarity of vertebrate cells. The stability and relative cell specificity of some IF polypeptides (eg cytokeratins: epithelial cells; desmin: skeletal mucle; glial fibrillary acidic protein: astrocytes; neurofilaments: neurons/axons) have led to their routine use as immunohistochemical cell lineage 'markers' in diagnostic pathology. Distinct biochemical features of the intermediate filament subtypes impart a 'static' *vs* 'plastic' character that is directly related to cell function. For example, extensive phosphorylation of the low, middle and high molecular weight neurofilament polypeptides stabilizes the axonal cytoskeleton such that 'hard-wiring' of the major CNS tracts will persist and remain fairly static. In contrast, IFs such as vimentin, GFAP, and nestin may, under physiologic and neoplastic conditions, disaggregate and reaggregate to allow for the plasticity necessary for cell proliferation and migration to occur. Nestin was first described as a major intermediate filament expressed by proliferating neuroepithelial stem cells and one of its functions is to promote the disassembly of vimentin for equal distribution to daughter cells during mitosis.

More recent work has identified nestin expression in non-neuroepithelial cells such as proliferating angiogenic endothelium during development and in neoplasia. In this issue, **Aihara *et al***,[1] (p. 1581) describe the expression of nestin in angiogenic endothelial cells and identify an endothelium-specific regulator in the first intron of the gene coding this intermediate filament protein. Extending the results of a prior study in which nestin was identified in proliferating vascular endothelial cells of malignant gliomas,[2] this group now demonstrates nestin expression in angiogenic capillaries of common non-neurogenic tumors such as lung, colon, stomach and cervical carcinomas, and in the microcapillaries of hemangioblastoma. Nestin was then shown to be expressed in cultured, proliferating, subconfluent human umbilical cord endothelial cells but not by quiescent confluent cultures. Finally, by comparing the effect of nestin regulators on gene expression and capillary tube formation, the authors localized a specific regulator in the first intron. This suggests that in proliferating endothelial cells, nestin is regulated differently from neural stem cells where the putative regulatory element is in the second intron. These findings suggest that nestin intermediate filaments may play a role in angiogenesis (and possibly in vascular stem cells) in addition to neuroepithelial stem cells. 'V-nestin' anyone?

References

1 Aihara M, Sugawara K, Torii S, *et al*. Angiogenic endothelium-specific nestin expression is enhanced by the first intron of the nestin gene. Lab Invest 2004;84:1581–1592.
2 Sugawara K, Kurihara H, Negishi M, *et al*. Nestin as a marker for proliferative endothelium in gliomas. Lab Invest 2002;82:345–351.

RET expression in papillary carcinoma

Oncogenic c-RET activation in thyroid tumors of follicular cell derivation is the result of chromosomal rearrangements. The rearranged RET forms, called RET/PTC (rearranged in transformation/papillary thyroid carcinoma), are the result of fusion of the RET tyrosine-kinase domain with the 5′-terminal regions of heterologous genes. Although RET/PTC is considered a molecular signature for papillary carcinoma, its reported prevalence varies broadly. Additionally, unrearranged c-RET expression has been identified in a highly variable proportion of papillary carcinoma samples and may also play a role in thyroid tumorigenesis. The reasons for the great variability in RET/PTC and c-RET levels in this disease are not well understood. There is an apparent geographic factor, as shown by comparing the prevalence of RET/PTC activation in various worldwide studies. However, much of the variability appears to be related to the sensitivity of the technique utilized to identify the rearrangement. In this issue, the paper by **Rhoden *et al***[1] (p. 1557) is the first detailed report of quantitative expression analysis of c-RET and its rearranged forms in papillary thyroid carcinoma. The authors extracted RNA from papillary carcinoma and normal thyroid samples and analyzed them by real-time quantitative RT-PCR for the expression of nonrearranged c-RET and the most common rearranged forms, RET/PTC1 and RET/PTC3. Their findings demonstrate that c-RET and RET/PTC levels are highly variable and exhibit distinct expression patterns. The variability is highest (up to about 100 000-fold) for RET/PTC1. This heterogeneity of expression helps explain the apparent inconsistencies in the reported detection rates and should be taken into account

for diagnostic purposes. Identification of this variability should also promote better understanding the role of c-RET activation in thyroid follicular cell tumorigenesis.

Reference

1 Rhoden KJ, Johnson C, Brandao G, *et al*. Real-time quantitative RT-PCR identifies distinct c-RET, RET/PTC1 and RET/PTC3 expression patterns in papillary thyroid carcinoma. Lab Invest 2004; 84:1557–1570.

Colorectal carcinogenesis: no more mousing around

The molecular insights gained into human colorectal carcinogenesis constitute one of the major triumphs of biomedical research in the last 30 years. Critical to the advance was identification of the *adenomatous polyposis coli (APC)* gene on human chromosome 5q21. *APC* regulates β-catenin degradation, and hence the ability of β-catenin to activate the nuclear cell cycle. Mutations in this gene are present in the majority of colorectal adenocarcinomas. Germline mutations in *APC* are responsible for the autosomal dominant familial adenomatous polyposis (FAP) syndrome. A murine model of FAP was identified in 1992, whereby a heterozygous germline mutation in *Apc, Apc*$^{Min/+}$, produced mice with multiple intestinal adenomas and adenocarcinomas. Unlike human FAP patients, however, *Apc*$^{Min/+}$ mice exhibit adenomas/adenocarcinomas predominantly in the small intestine. The same has been true for subsequent murine lines with other mutations in *Apc*. A key feature of both human FAP and the murine *Apc* mutants is that the germline mutations are heterozygous only; a wild-type allele also remains. In critical support of the Knudsen 'two-hit' paradigm, an acquired somatic loss of wild-type APC function (either by loss-of-heterozygosity—LOH, or by mutation *per se*) leads to adenoma development, and eventual adenocarcinoma in those adeonomas that acquire further chromosomal derangements. In humans, the 'second hit' to *APC* produces predominantly colorectal adenomas/adenocarcinomas; in mice it produces predeominantly small intestinal adenomas/adenocarcinomas. The reasons remain unknown.

In this issue, **Colnot *et al***[1] (p. 1619) report a new murine *Apc* model that develops colorectal neoplasia. A Cre-loxP strategy was used to delete exon 14 of *Apc*. The *Apc*$^{\Delta 14/+}$ mice rapidly developed colorectal cancers, detectable by rectal prolapse (see cover photograph). All neoplastic lesions, adenomas or adenocarcinomas, revealed *Apc* LOH in the remaining wild-type allele, and thus loss of *Apc* gene expression. The expected changes in β-catenin accumulation and altered cell cycle control were fully expressed. The authors also show that the microbial microenvironment is critical for expression of the colorectal cancer phenotype. Hence, this mouse model provides substantive new opportunity to advance understanding of the molecular pathogenesis of colorectal cancer.

Reference

1 Colnot S, Niwa-Kawakita M, Hamard G, *et al*. Colorectal cancers in a new mouse model of familial adenomatous polyposis: influence of genetic and environmental modifiers. Lab Invest 2004;84:1619–1630.

Fetal microchimerism in the adult human female: generation of male hepatocytes

The first report of bone marrow-derived stem cells generating mature hepatocytes within the mammalian liver was in 1999, based on rodent liver work. The exciting possibility that bone marrow-derived stem cells could support restitution of the human liver was reported in 2000, whereby female recipients of male bone marrow transplants or male recipients of female donor livers were found to have Y chromosome-containing hepatocytes. Initial enthusiasm for these findings has been tempered by a series of reports over the next 4 years documenting that such restitution occurs only at extremely low levels (ie, one hepatocyte per $>100\,000$ hepatocytes), if at all, and by a 2002 report that bone marrow-derived cells are quite capable of fusing with recipient somatic cells to generate an artefactual phenotype of Y chromosome expression. The debate has continued unabated as to whether restitution occurs at higher rates, and whether it is a fusion artefact or true 'transdifferentiation' of stem cells into hepatocytes. Hanging in the balance is whether stem cell therapy will be of eventual value for treatment of human liver disease.

A natural phenomenon whereby 'exogenous' stem cells enter the circulation is pregnancy. So-called fetal chimerism is well-documented, and can be tracked most easily by identifying Y chromosome-containing cells derived from male fetuses within the maternal circulation, especially immediately after parturition. Such cells can persist in maternal tissues for decades, including the liver. Y chromosome-containing cells and male DNA can be found in livers of mothers of male children, regardless of whether the mothers' livers are diseased or normal. What has not been determined is the cellular phenotype of these conceptus-derived cells. A separate consideration is whether the presence of fetal cells in the maternal liver engenders autoimmune disease.

Stevens *et al*[1] (p. 1603) now report that liver biopsies from adult human mothers of male children contain epithelial cells with a hepatocyte phenotype, in the absence of evidence of cell fusion. Liver biopsies obtained for evaluation of viral hepatitis, autoimmune hepatitis, or steatohepatitis were

examined for X and Y chromosome content. An exceedingly low number of Y chromosome-containing cells were identified. Only 25% of 28 female liver biopsies contained male cells. On average, there was about one male cell per 30 000 host cells (25 mm^2) in patients with primary biliary cirrhosis, or the size of one generous liver biopsy histologic section. This value was 0.17 male cells per 30 000 cells in hepatitis C infection, and 0.35 male cells per 30 000 cells in other conditions. About half of these cells were hepatocytes, on the basis of Cam5.2 immunoreactivity, absence of CD45 immunoreactivity, and presence in the parenchyma. The possibility that the Cam5.2-positive cells were progenitor cells rather than mature hepatocytes was not excluded, but the epithelial nature of these cells was established. No male cells were found within bile ducts or endothelium. Importantly, none of the Y chromosome-containing cells contained more than one X chromosome, effectively excluding fusion of fetal XY cells with maternal XX cells as an explanation for the findings. As a control group, liver biopsies from females 8 and 24 months old were found to contain no male cells.

These findings strengthen the concept that fetal microchimerism can give rise to mature hepatocytes within the maternal liver, persisting for decades after pregnancy. This study does not provide information on whether the host immune system is adversely (or favorably) affected by the presence of such partially foreign cells. What it does establish is that there is a low background population of Y chromosome-containing hepatocytes in mothers of male children. This information is critically important for proper interpretation of studies regarding stem cell restitution of the human liver, as they may be confounded by fetal microchimerism.

Reference

1 Stevens AM, McDonnell WM, Mullarkey ME, *et al*. Liver biopsies from human females contain male hepatocytes in the absence of transplantation. Lab Invest 2004;84:1603–1609.