Laboratory Investigation (2004) 84, 811–813

www.laboratoryinvestigation.org

INSIDE LAB INVEST

Laboratory Investigation (2004) **84,** 811–813. doi:10.1038/labinvest.3700119

CD44 expression in prostate cancer: it depends on how you splice it

CD44 is a polymorphic family of transmembrane glycoproteins involved in homotypic cell, cell–matrix, and cell–cytoskeletal interactions. The standard form of CD44 (CD44s) has a widespread tissue distribution. While some normal epithelial cells express splice variants of this adhesion molecule in a tissue-specific manner, many types of cancer express novel variant isoforms, reflecting deregulated mRNA splicing, via duplication or deletion of variant exons. Isoforms are identified by the variant numbers, eg, v6. The literature on the role of CD44 in cancer is rich and complex. Normal prostate tissue expresses CD44s and a number of variant isoforms. In prostate cancer, the immunohistochemical expression of CD44s and CD44v6 are lost as the tumor grade increases, but CD44v7 is overexpressed. In contrast, several other forms of cancer exhibit amplification of CD44v6. In this issue, **Omara-Opyene *et al*** (p. 894) demonstrate CD44 variant expression patterns that are unique to prostate cancer and show functional significance. In their study, CD44v7–v10 was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in prostate cancer tissues and lymph node metastases, but not in benign prostatic tissue. Silencing the expression of CD44v9, the longest sequence within the CD44v7–v10 region, using RNA interference technology, significantly reduced the invasiveness of prostate cancer cell lines *in vitro*. This decrease in invasiveness was observed for both PC3 M cells and a derivative cell line that is known to be more invasive than the parental line, as it constitutively expresses the G-protein $G_s\alpha$. Furthermore, the authors also showed that $G_s\alpha$ cells overexpressed CD44v9 in comparison to PC3 M cells, which suggests a novel link between signal transduction and cell adhesion marker expression. These findings open new pathways of investigation for gene therapy with RNAi against CD44 variants or for design of small molecule inhibitors.

References

1 Omara-Opyene AL, Iczkowski KA, Qiu J, *et al*. Prostate cancer invasion is influenced more by expression of a CD44 isoform including variant 9 than by Muc 18. Lab Invest 2004;84:894–907.

Muscling in on prion disease

The human prion diseases range from classic, sporadic Creutzfeldt–Jacob disease (CJD) to the more recently described fatal familial insomnia and variant CJD (vCJD). Most notable for their devastating and largely untreatable effects on the central nervous system, these diseases are defined by accumulation of abnormal (misfolded) forms of prion protein (PrP). The association of vCJD with an outbreak of bovine spongiform encephalopathy (BSE) in Great Britain during the last decade has accelerated efforts to understand the production, processing and potential effects of prion protein deposition outside the central nervous system. In this issue, **Furukawa *et al*** (p. 828) report that long-term chloroquine treatment of Syrian hamsters results in accumulation of abnormal, mildly protease-resistant PrP in skeletal muscle. However, no infectivity was observed when muscle homogenates were inoculated intracerebrally, suggesting that this peripherally accumulated prion has distinct biochemical and biological properties. The study has potential clinical relevance for emerging diagnostic and therapeutic approaches in the human prion diseases. First, human CJD is being treated with quinacrine, another antimalarial drug that is lysosomotrophic and increases the pH of lysosomes. Second, evidence is emerging that some as yet undetermined form of abnormal, protease-sensitive PrP accumulates in skeletal muscle in CJD patients. If the latter is confirmed, muscle biopsy may allow for early diagnosis of CJD so that treatment can begin at the earliest possible time during the course of the disease. This makes it necessary to know the range of conditions that result in accumulation of abnormal PrP in skeletal muscle. Some investigators have proposed that skeletal muscle may be useful for tracking the effectiveness of CJD treatments. The results of this study suggest that lysosomotrophic drugs may create a false-positive abnormal PrP signal that is not CJD-related.

References

1 Furukawa H, Doh-ura K, Sasaki K, *et al*. Accumulation of prion protein in the muscle fibers of experimental chloroquine myopathy: *in vivo* model for deposition of prion protein in non-neuronal tissues. Lab Invest 2004;84:828–835.

Domino liver transplantation for familial amyloid polyneuropathy

Arrival in the surgical pathology cutting suite of an intact organ essential for life took some getting used

to in the early days of organ transplantation, especially heart and liver for which there is no paired organ. Nevertheless, the customary end-stage nature of the resected organ provides assurance to the prosecting pathologist that placement of a new organ in the patient was the only option for sustained life. It is therefore even more intriguing to receive a normal liver explant as the outcome of a liver transplant operation. There is one condition in which this occurs: familial amyloid polyneuropathy (FAP). This disorder is an autosomal dominant neurodegenerative disorder resulting from systemic extracellular deposition of mutated transthyretin amyloid fibrils in connective tissue. Brain and liver are singularly excluded from this deposition. In contrast, the peripheral nervous system is devastated by deposition of amyloid fibrils in the endoneurium. The onset of clinical symptoms generally occurs before age 40 years, and consists of progressive and severe sensory, motor, and autonomic polyneuropathy. Untreated, the disease relentlessly progresses to severe disability and death. The only treatment that halts the progression of disease is liver transplantation, as the liver is the culprit organ, responsible for synthesis and secretion of approximately 90% of plasma transthyretin. The first liver transplant performed for FAP was in 1990, and by now more than 800 patients have been transplanted worldwide. Given the shortage of organs for liver transplantation, the FAP patient has been used as both living donor and as recipient. The FAP patient receives a liver allograft, and donates his/her histologically 'normal' native liver to another recipient in a unique operative sequence designated domino liver transplantation (DLT). This procedure then generates the striking issue of transplanting a liver graft that is producing a pathogenic protein (mutated transthyretin) into a recipient patient. The question is: how long will it take to develop the peripheral neuropathy in the recipient? This question has now been addressed in a major study by **Sousa *et al*** in this issue (p. 865), whereby *de novo* amyloid deposition and toxicity was examined in the skin and nerve of recipients of FAP livers up to 7 years after DLT. These Portuguese authors took advantage of the fact that the greatest concentration of FAP patients and patient kindreds is in Portugal. In all, 15 patients were studied; 13 male and two female. The authors found that transthyretin deposition occurred in the skin 3 years after transplantation, either as amyloid or as protein aggregates. In one patient only, fibrillar transthyretin was present in the epineurium as scarce deposits 6 years after DLT. Nerve biopsies from DLT recipients had no FAP-related neuropathy. These findings indicate that transthyretin amyloid formation definitely occurs in recipients of FAP livers, and that transthyretin of liver origin can cross the blood–nerve barrier. Indeed, the deposition of amyloid within 3 years suggests an accelerated rate of deposition when compared to native FAP patients. However, deposition of amyloid in the nerves of DLT recipients is scarce and does not appear to produce active pathology in the early years after DLT. The longer term effect of DLT still remains unknown, and the authors propose that DLT using FAP livers should continue to be viewed as an experimental procedure requiring careful surveillance and follow-up.

References

1 Sousa MM, Ferrão J, Fernandes R, *et al*. Deposition and passage of transthyretin through the blood nerve barrier in recipients of familial amyloid polyneuropathy livers. Lab Invest 2004;84:865–873.

Blood mitochondrial DNA: the HAART of the matter

Highly active antiretroviral therapy (HAART) revolutionized AIDS therapy and saved many lives. However, this great success carries a price. Patients often experience significant side effects including lactic acidosis, hepatic steatosis, myopathy, cardiomyopathy, peripheral neuropathy, pancreatitis and the lipodystrophy syndrome. These disorders have been associated, at least partly, with the actions of nucleoside analog reverse transcriptase inhibitors (NRTIs), which are believed to cause mitochondrial toxicity. The latter is related to the inhibition of DNA polymerase-γ, an essential enzyme for the replication of mitochondrial DNA (mtDNA). Since depletion of mtDNA was recently reported to be a marker of NRTI toxicity, several studies examined whether quantification of mtDNA in peripheral blood cells could be a more sensitive and earlier indicator of the mitochondrial toxicity, with conflicting results. Inside this issue, **Chiappini *et al*** (p. 908) investigated the long-term toxicity of HAART in 157 consecutive outpatients to determine whether the mtDNA to nuclear DNA (nDNA) ratio in peripheral blood mononuclear cells can be used to screen for mitochondrial toxicity in routine clinical practice. The mtDNA to nDNA ratio in peripheral blood mononuclear cells was analysed by real-time quantitative PCR. While this ratio was lower in HIV-infected patients than in noninfected controls, there was no apparent difference in the mtDNA/nDNA ratio between HAART-treated and untreated HIV-infected patients, nor did this ratio have a significant relationship to clinical symptoms or lactate level. This study did not support measurement of the mtDNA to nDNA ratio in peripheral mononuclear cells as a marker of mitochondrial toxicity of HAART.

References

1 Chiappini F, Teicher E, Saffroy R, *et al*. Prospective evaluation of blood concentration of mitochondrial DNA as a marker of toxicity in 157

consecutively recruited untreated or HAART-treated HIV-positive patients. Lab Invest 2004; 84:908–914.

Molecular progression of gastric and colorectal cancer

The clarion call for the 21st century pathologists is to understand human disease at the molecular level, as opposed to the structural level. While there appears to be no immediate threat to the continued use of light microscopes for diagnostic purposes, pathologists are nevertheless charged with intense molecular investigation of neoplastic diseases, in particular, for diagnostic and prognostic purposes. Chromosomal instability and microsatellite instability are two major molecular pathways for development of cancer. Two papers in this issue, by **Yao *et al*** (p. 915) and by **Lee *et al*** (p. 884), provide valuable new information on the molecular changes in gastric and in colorectal cancer, respectively. In the first paper, the DNA mismatch repair system (MMR) was examined in a series of 14 human gastric cancer cell lines *in vitro*, to determine whether variations in the levels of MMR proteins or mutations in the main DNA MMR genes were associated with microsatellite instability status. As the MMR system is essential for DNA replication fidelity, the presence of high-level microsatellite instability might be a significant harbinger of malignant behavior. The authors found that marked decreases in the expression levels of key MMR proteins (hMLH1, hPMS2, hPMS1) was associated with high levels of MSI mutations in the gastric cancer cells. Plausible next steps for this line of investigation are to: determine how the status of the MMR system influences the behavior of these gastric cancer cell lines when implanted into appropriate experimental animals *in vivo*; and to determine whether defects in the MMR system can be found to have both pathogenetic and prognostic importance *in vivo*. In the second paper, a third molecular pathway for cancer development was examined: tumor suppressor gene inactivation via CpG island hypermethylation. A total of 271 tissue specimens from the human colon were examined, ranging from normal colon, colon adenoma, and colorectal cancer, with the goal of studying not just specific candidate genes, but rather of studying concordant methylation of multiple genes. In total, 12 critical genes were studied, known to be involved with cell cycle regulation (*COX-2, p14, p16*), DNA repair or protection (*hMLH1, MGMT, GSTP1*), signal transuction (*APC, RASSF1A*), apoptosis (*DAP-kinase*), angiogenesis (*THBS1*), and metastasis and invasion (*E-cadherin, TIMP-3*). A stepwise increase in methylation was observed, in comparing normal to adenomatous to cancerous tissue. The total number of methylated genes per tumor showed a continuous, nonbimodal distribution in colon adenoma or cancer. Interestingly, such methylation was more prevalent in proximal colon cancer specimens than in distal colon cancer. These data indicate that CpG island methylation is an early and frequent event during colorectal carcinogenesis, may be a third major contributor to colorectal carcinogenesis, and may be particularly important in the more proximal colon. Collectively, these two papers help push the molecular study of alimentary tract cancer forward.

References

1 Yao Y, Tao H, Kim JJ, *et al*. Alterations of DNA mismatch repair and microsatellite instability levels in gastric cancer cell lines. Lab Invest 2004;84:915–922.
2 Lee S, Hwang KS, Lee HJ, *et al*. Aberrant CpG island hypermethylation of Multiple genes in colorectal neoplasia. Lab Invest 2004;84:884–893.