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Proteomic analysis of archived formalin-fixed paraffin-embedded human tissue

Comprehensive molecular analysis of archived human tissues is rapidly becoming a requisite standard for high-throughput exploration of the molecular basis of disease. While analysis of frozen human tissue has been the starting point for exploration of the genome, transcriptome, and proteome, obtaining such tissue samples is arduous and sometimes impossible, owing to the priority of performing the clinical tissue assessment before release of excess tissue for research purposes. However, formalin-fixed paraffin-embedded (FFPE) human tissue is broadly available to investigators (once the hurdles of consent and IRB approval have been successfully addressed). Moreover, linkage of molecular analysis to clinical information, especially outcomes data, is a powerful driver of hypothesis-testing and validation. Successful inroads have been made into obtaining valid genomic data from FFPE human tissue. However, to date proteomic analysis of peptide extracts has been reported only in preliminary form.¹ In this issue are two reports of successful extraction of peptides from FFPE human tissue with subsequent proteomic analysis. In the first, **Chu *et al***² (p. 1416) developed a rapid method for extracting proteins and nucleic acids directly from a single formalin-fixed paraffin-embedded or frozen tissue section, without destroying tissue morphology. Ultrasound and microwave treatment in the setting of an appropriate extraction buffer facilitated release of macromolecules, which could be used directly without further purification for SDS-PAGE analysis, immunoblotting, protein array, mass spectrometry, PCR and RT-PCR reactions. Simultaneously, this extraction protocol serves as an antigen retrieval treatment, enabling processing of the same tissue section for histopathology, including routine staining, immunohistochemistry, and *in situ* hybridization. Hence, both histological assessment and comprehensive molecular analysis can be performed on a single tissue section, whether it is from a frozen or fixed tissue specimen. Although these authors demonstrated that the peptide extracts were amenable to analysis by mass spectrometry, a key step that Chu *et al* did not do was specifically identify peptides so extracted. This has been accomplished by **Crockett *et al***³ (p. 1405), who employed protease enzymatic cleavage for extraction of peptides from FFPE specimens, followed by proteomic analysis via liquid chromatography

and mass spectrometry (MS/MS). In this study, a human lymphoma cell line was either analysed as a fresh cell lysate, or as an extract from 3-year-old formalin-fixed paraffin-embedded cell blocks. Although only 94 proteins could be identified on a single extract from a fresh cell lysate, and 88 proteins from a FFPE cell block, these numbers increased to 514 and 324 proteins identified, respectively, when data were compiled from 10 replicate extractions of each sample type. There were 263 common peptides identified. Western blot analysis and immunofluorescence microscopy confirmed the presence of selected identified proteins, and the functional significance of one protein (Protein Kinase C η) was validated using a PKC-inhibitory peptide, which resulted in lymphoma cell death *in vitro*. These authors have therefore demonstrated that large sets of proteins can be identified from FFPE specimens, enabling testing of their potential biological significance. As noted by both sets of authors, these reports have major implications for proteomic analysis of the vast repositories of archived paraffin-embedded human tissue, and the opportunity to advance molecular understanding of human disease.

References

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- 2 Chu WS, Liang Q, Liu J, *et al*. A nondestructive molecule extraction method allowing morphological and molecular analyses using a single tissue section. *Lab Invest* 2005;85:1416–1428.
- 3 Crockett DK, Lin Z, Vaughn CP, *et al*. Identification of proteins from formalin-fixed paraffin-embedded cells by LC-MS/MS. *Lab Invest* 2005;85:1405–1415.

CNS expression of Fussel 18: a new way to Ski (with a Sno clone)

TGF β signaling is mediated by Smad family proteins, which are distributed in the cytoplasm and nucleus. Following TGF β binding to its receptor, Smad 2 and Smad 3 become phosphorylated and oligomerize to form Smad 4. Such Smad complexes accumulate in the nucleus and activate transcription of TGF β -target genes. Negative regulators of Smad function include Smad 7, Ski, and Sno. An eight base-pair palindromic sequence was initially identified as a high-affinity binding site for Ski but this is an indirect binding process, which also requires Smad 3 and 4.

Inside this issue, **Arndt et al**¹ (p. 1330) describe the cloning and functional characterization of a novel Ski homolog, called Fussel 18 (Functional Smad-suppressing element on chromosome 18). The gene was identified by searching the human genome (NCBI), EST, and IMAGE gene banks using BLAST. These searches revealed partial or full-length *ski* and *sno* clones in addition to a third group, which was assembled and designated as *fussel 18*. The complete cDNA sequence is provided and the deduced amino-acid sequence shows 29–37% homology with Ski protein.

In contrast to the rather broad organ and tissue distribution displayed by other *ski* and *sno* gene family members, *in situ* hybridization studies with murine embryos and RT-PCR analysis of adult animals showed that *fussel 18* is only expressed in the cerebellum and spinal cord. Immunofluorescence studies revealed strong Fussel 18 reactivity in cerebellar glomeruli of the internal granule cell layer. Here, incoming signals from the spinal cord and elsewhere are integrated for proper sensory and motor coordination. These findings suggest that Fussel 18 may play a role in proper cerebellar and spinal cord development and function, while abnormal Fussel 18 could be associated with neurodevelopmental or neoplastic conditions. Further studies in this area may provide significant insights into TGF α regulation in the nervous system and may explain the way people are either 'ski'ing or falling down.

Reference

- 1 Arndt S, Poser I, Thomas Schubert, *et al*. Cloning and functional characterization of a new Ski homolog, Fussel-18, specifically expressed in neuronal tissues. *Lab Invest* 2005;85:1330–1341.

Hypertrophied cardiac myocytes and their ILK

Much attention is given to the interconnectivity of cardiac myocytes, one to another. The relationship of cardiac myocytes to the extracellular matrix also merits close examination. Attachment of myocytes to the immediately adjacent extracellular matrix is mediated by transmembrane receptors, of which integrins feature prominently. Integrins link the extracellular matrix (for example, fibronectin) to the intracellular cytoskeleton, thereby promoting structural integrity for this tissue. Just as important is the role of integrins in transmitting information from the extracellular matrix to intracellular signaling pathways (outside-in signaling) and in modulating integrin affinity for the extracellular matrix in response to intracellular signals (inside-out signaling). A case in point is cardiac hypertrophy, in which hypertrophy of cardiac myocytes is accompanied by marked changes in extracellular matrix composition, the development of fibrosis, and impairment in contractile function, which leads

paradoxically to heart failure. Integrin-dependent pathways are critical in mediating the hypertrophic response to mechanical stimuli associated with cardiac myocyte strain, and synergize with other hypertrophic stimuli such as the alpha-adrenergic agonist phenylephrine. In this issue, **Chen et al**¹ (p. 1342) examined the role of the integrin-linked-kinase (ILK) in mediating integrin signaling pathways that regulate cardiac myocyte hypertrophy and resistance to apoptosis. In other cell types such as fibroblasts, ILK is involved in cell adhesion, motility, and survival. ILK is an ankyrin repeat-containing serine-threonine protein kinase that binds to the cytoplasmic domain of $\beta 1$, $\beta 2$, and $\beta 3$ integrin subunits. It forms a tripartite complex with two other intracellular proteins, PINCH1 and alpha-parvin (PARVA), designated IPAP1, assembly of which is required for ILK kinase activity and promotion of cell growth, motility, and resistance to apoptosis. Since fibronectin has been shown to induce cardiac myocyte hypertrophy *in vitro*, Chen *et al*¹ hypothesized that fibronectin and phenylephrine stimulation might regulate the expression, localization, and function of the IPAP1 complex in cardiac myocytes. They demonstrated that localization of IPAP1 complex proteins to costameres in cardiac myocytes is stimulated synergistically by fibronectin and phenylephrine, and that a dominant-negative mutant of the PINCH-binding N-terminus of ILK (ILK-C) prevented costamere association of ILK and PARVA, but not PINCH. Fibronectin and phenylephrine induced hypertrophy, which was stimulated by low levels of ILK-C. In support of the hypothesis, high expression of ILK-C repressed fibronectin and phenylephrine-induced hypertrophy, and induced marked apoptosis of cardiac myocytes. The authors conclude that the IPAP1 complex plays a critical role in mediating the integrin signaling pathways that regulate cardiac myocyte hypertrophy and resistance to apoptosis. The key role of ILK is especially clear, and it may be that ILK acts as a scaffold for the assembly of either pro- or antihypertrophic intracellular signaling complexes, depending on extracellular stimuli and other intracellular mediators. The relationship between cardiac myocytes and their extracellular matrix may thus play a critical role in the response of the heart to injury, or the perpetuation of injury once it occurs.

Reference

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A new orthotopic prostate cancer model

Therapy-resistant metastasis is the leading cause of human prostate cancer death. The development of

new, effective therapies is critically dependent on the availability of clinically relevant *in vivo* models, in part because the molecular and cellular mechanisms involved in the progression of the disease remain poorly understood. Current animal models consist of implanting xenografts of well-established human prostate cancer cell lines, which have been adapted to *in vitro* growth into immunodeficient mice. However, the xenografts are of highly anaplastic prostate cancer cells, which represent the extreme end of highly advanced cancers. Such xenografts are not associated with deposition of original tumor stroma, which is now recognized as a crucial factor in the pathogenesis of cancer metastasis. Accordingly, various groups have attempted to develop more relevant models based on xenografts of primary prostate cancer tissue in immunodeficient mice. Unfortunately, xenografts to subcutaneous sites result in exceedingly low tumor take rates, and have only been successful in cases of highly advanced malignancies. Furthermore, the subcutaneous compartment is a non-orthotopic graft site and does not mimic the original tumor microenvironment, nor does it allow full expression of metastatic potential. Therefore, development of a model that could efficiently and reproducibly allow generation of orthotopic xenografts would be of great interest to prostate cancer researchers.

In this issue, **Wang *et al***¹ (p. 1392) describe the development of a new *in vivo* model system for metastatic prostate cancer. They employed human prostate cancer tissue which was grafted into

testosterone-supplemented male NOD-SCID mice, into the renal subcapsular space. Samples of metastatic tissue were then regrafted in mouse anterior prostates and their growth and spread examined. The authors were able to isolate a metastatic subline, PCa1-met, from lymph nodes, and showed that orthotopic grafting of PCa1-met tissue into naive NOD-SCID mouse hosts led to metastases to multiple organs, including bone. Histopathological analysis showed strong similarity between orthotopic grafts and their metastases, and spectral karyotyping showed few chromosomal alterations in the PCa1-met subline. This study indicates that, unlike primary cancer tissue, transplantable renal subcapsular xenografts of human prostate cancer tissue in NOD-SCID mice can be successfully grown in an orthotopic site.

The orthotopic prostate cancer metastasis model developed in this study should be useful for investigations of the mechanisms underlying prostate cancer metastasis, and may also have therapeutic applications. The methodology used to develop this model may also be suitable for development of orthotopic metastatic cancer models of other types of human cancer.

Reference

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