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Challenges with Human Tissues for Gene and Protein Target Validation

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The mission of the Target Validation & Molecular Pathology group is to validate scientifically qualified targets very early in the exploratory phase as well as to support downstream projects in drug development. The use of normal and diseased human tissues is critical to this process. Target validation requires gene expression data (RT-PCR) as well as cellular localization data obtained via in situ hybridization (ISH) or immunohistochemistry (IHC) consistent with the proposed disease hypothesis. This mission, therefore, required the establishment of an Aventis biorepository.

The tissue biorepository consists of specific human and animal tissues, cells, and RNAs. Obtaining human tissues has proven to be a difficult challenge. This task is a shared responsibility between Molecular Pathology and our Disease Group collaborators within Global Aventis. We have utilized contract templates and partnered with Legal and Business Development. Our sources for human tissues are medical centers, hospitals, institutions, and commercial 3rd party vendors. The contract types have varied from Research Collaborations to Fee-For-Service to Material and Services Agreements. We are able to complete contracts on about half of those sources contacted. For example, we have completed contracts on 18 of 39 potential sources to date. Currently, our biorepository consists of approximately 3400 human and 1600 animal biospecimens. In addition, we have about 10,000 tissue slides at any one time. We continue to search for sources to fill remaining gaps and to maintain our biorepository. Our current gaps are normal tissues, specific disease categories and increased numbers to meet 2003 tissue panel objectives.

There are a myriad of issues that can affect the successful establishment of a quality biorepository, especially for human biospecimens. Issues that impact completion or execution of contracts include lack of quality tissues, inadequate resources, intellectual property, indemnification/liability insurance, IRB rejection, business strategy change, lack of informed consent or data use authorization, and insufficient budget. After acquisition of biospecimens, we assess their quality. We verify (1) the concordance of histologic features with normal or disease categories, (2) the integrity, quality and content of sample RNA, and (3) the integrity of protein. The percentage of samples passing quality assessments varies, but 33% is common for lung/bronchus surgical specimens and 20% for CNS autopsy tissue. Additional issues include biomarker signature patterns, sample size limitations and balancing QC standards with sample availability. Compliance with evolving privacy law presents an additional challenge concerning data sets associated with obtaining human tissue. US privacy law under HIPAA (Health Insurance Portability & Accountability Act) addresses research using de-identified data sets, limited data sets and data use agreements, with IRB/Privacy Board Waivers, and with authorization [1]. We are in discussions with the Aventis Global Privacy Task Force to take the limited data set approach for human tissue acquisitions in 2003. We are also investigating the impact of European privacy laws for future Aventis global viewing of European and US instances of our biorepository databases.

Our infrastructure includes a biorepository and database; histology QC and histopathology analyses; ISH/IHC and digital bioimaging analyses; RNA isolation and cDNA synthesis/QC; RT-PCR/TaqMan® (TM) analyses; and laser capture microdissection for expression profiling of specific cell types. These technologies are conducted on normal and diseased tissue or cell panels. Figure 1 illustrates the varied tissue specificity and expression levels of two closely related genes across a panel of normal human tissues as determined by RT-PCR analyses. The mRNA levels are relative to Beta-2 microglobulin, the endogenous control. Figure 2 illustrates the specific ISH expression of a low copy G-Protein Coupled Receptor (GPCR) target gene within a human immune cell preparation.

In summary, despite the challenges with human tissue, we have been successful in obtaining critical biospecimens and have made significant impact on target validation in the early exploratory stages and will impact projects at later stages in the drug discovery pipeline in the near future.

[1] Daniels, J. HIPAA for Drug & Device Companies. Philadelphia, December 9-11, 2002. Institute for International Research, USA.

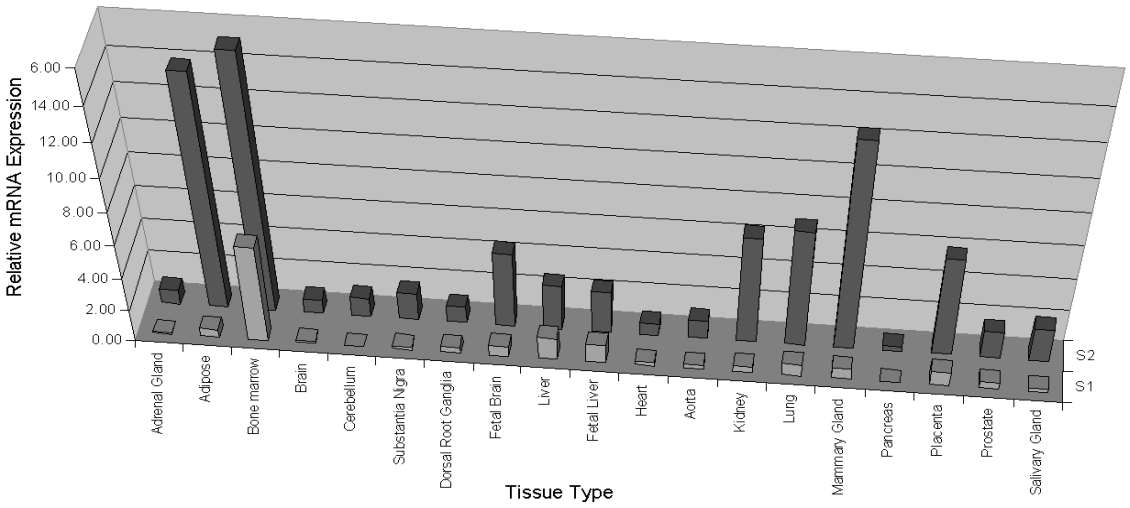

Figure 1. Tissue specificity varied between two closely related GPCR targets (RT-PCR). Note large difference in relative mRNA expression in adipose, bone marrow, and mammary gland tissues.

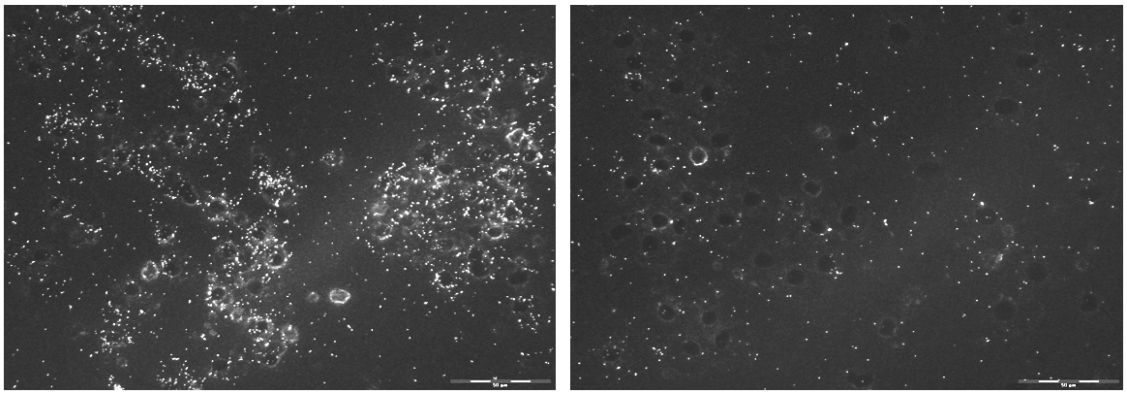

Figure 2. Left panel illustrates ISH darkfield antisense signal of GPCR target gene expressed in human immune cell cytospin preparation. Right panel illustrates background sense signal. 40X.

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