Track: disease biomarkers

Development and optimization of a procedure for extracting proteins from fixed archival tissues and assessment of its potential for biomarker discovery in human and veterinary medicine

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Summary

A protocol for generating high quality protein extracts from fixed tissues was developed and applied to archival samples from human and veterinary pathology departments. The results obtained in terms of compatibility with disease biology and literature data are presented.

Abstract

Fixed tissue repositories are an ideal information mine for biomarker discovery, with their vast collections of diseased tissues and associated clinical records describing diagnosis, prognosis, therapy, and outcome. Unfortunately, fixation hampers protein extraction, posing considerable limitations to proteomic investigations. In this scenario, and building on the existing literature, several methods were combined and refined to enable extraction of high-quality, full-length proteins from the fixed specimens, leading to the definition of an optimized protocol with unprecedented performances. In order to test its ability to provide meaningful results in terms of differential disease traits and enable biomarker discovery, the optimized method was applied to sample repositories stored in pathology departments. Specifically, diseased human, canine and sheep tissues were retrieved and subjected to the optimized extraction procedure, followed by established proteomic pipelines consisting of electrophoresis, image analysis, mass spectrometry, and data processing by ontology and pathway analysis. The method enabled gel-based proteomic investigations of archival tissues, performing better than commercially available systems. Differential proteins were successfully identified in all cases, and these were clearly related to the disease biology. In conclusion, biomarker discovery strategies based on proteomic investigations can be successfully performed on fixed archival tissues by means of this optimized extraction protocol.