Proteomics of human cancer tissues and cells

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Proteomic analysis of cancer tissues and cells provides valuable information to identify promising targets for cancer diagnosis, prognosis and therapy. Novel strategies have emerged to optimize the workflow of tissue procurement, and tissue and cell selection, and to improve protocols for the extraction of protein from fresh, frozen and paraffin-embedded tissue. Moreover, in the context of advanced approaches to proteomics, mass spectrometry and array-based technologies strongly contribute to protein profiling of cancer tissues and cells.

The focus of this review is the methods by which all the steps of a proteomic investigation on human-cancer tissue (from choice of the experimental model to validation of candidate biomarkers) should be performed, paying particular attention to recently developed strategies. The review also presents an overview of the most recent high-throughput proteomic studies in cancer research.

Keywords: Array-based proteomic technology; Biomarker; Candidate biomarker; Cancer cell; Cancer research; Cancer tissue; Mass spectrometry; Protein profiling; Proteomic technology; Shotgun proteomics

Abbreviations: AbM, Antibody-based microarray; DIGE, Differential in-gel electrophoresis; DMSO, Dimethyl sulfoxide; FFPE, Formalin-fixed paraffin-embedded; GEP, Gene-expression profiling; IMS, Imaging mass spectrometry; IHC, immunohistochemistry; iTRAQ, isobaric tag for relative and absolute quantitation; LCM, Laser-capture microdissection; MS, Mass spectrometry; MRM, Multiple-reaction monitoring; RPPA, Reversed-phase protein microarray; TMA, Tissue microarray; 2D-PAGE, Two-dimensional polyacrylamide gel electrophoresis

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1. Introduction

The past decade has seen a dramatic increase in the use of high-throughput techniques, which can provide quantitative or semi-quantitative data about a large number of variables for each sample in a short time.

Gene-expression profiling (GEP) is by far the most successful technique of this kind, allowing simultaneous detection of almost all RNA transcripts within one or two days, or even hours with the latest devices. The success of GEP is based on the availability of robust platforms, as well as the requirement for only small quantities of input samples (RNA). GEP has now been applied to almost any cancer entity, including leukemia and lymphoma in the late 1990s [1,2] and then progressively extending to other cancer types (including breast, prostate, colon and lung, to name the most common) [3–6]. The main outcome of these studies has been the definition of molecular entities, which may or may not overlap with classical pathological entities, and to develop good algorithms for the determination of a patient's diagnosis and prognosis.

Unluckily, GEP did not bring the advent of new therapies, a much expected output of high-throughput techniques. What caused this partial failure of GEP is the intervention of dynamic processes in the biology dogma ''DNA -> RNA -> protein''. In the end, it is the protein dynamics that determine the behavior of a cell, including the capacity to multiply disproportionately, invade neighbors and eventually kill its host. As a result, most drug targets are proteins, and in proteins resides some of the hope to counteract the misbehavior of cancer cells. From a strategic point of view, intelligence work is vital to win any war. It is therefore logical to try and determine the ''proteomic soul'' of a cancer sample in order to aim at a weak point.

Usually, a set of experiments that involves the use of a proteomic technology follows a more or less repeatable path (Fig. 1) that involves:

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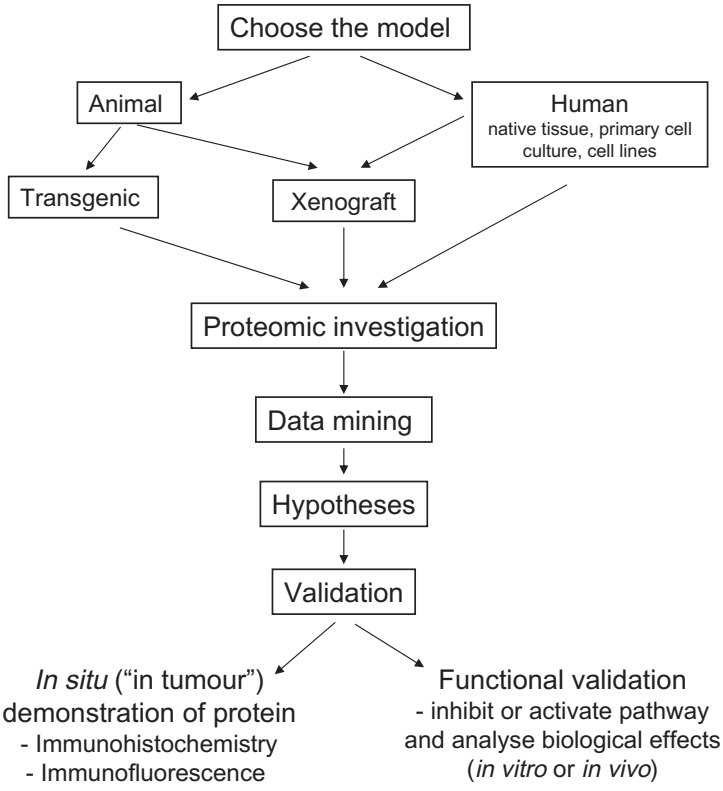

Figure 1. The typical experimental path for proteomic analysis of cancer tissues and cells, which involves: a) choice of the experimental model; b) proteomic analysis; c) data mining; d) generation of hypotheses; and, e) validation or confutation of proposed hypotheses.

(1) choice of the experimental model;
(2) proteomic analysis;
(3) data mining;
(4) generation of hypotheses; and,
(5) validation or confutation of proposed hypotheses.

This algorithm provides the necessary scientific basis for pre-clinical and clinical studies of new therapeutic options.

Since no perfect or near-perfect proteomic technique has emerged, applications to cancer research are still confined to a niche. The main factors that determine this are the requirement of large quantities for input samples, rather low reproducibility of assays, time-consuming technical procedures, low sensitivity of detection, and the presence of very highly abundant proteins obscuring the presence of those of lower abundance. Most of these drawbacks usually refer to classical 2D-PAGE proteomics, and possible solutions can be found by at least three strategies, which we discuss in the following paragraphs:

(1) improve the availability of tissues;
(2) improve technical automation; and,
(3) develop alternative technologies at a reasonable cost.

2. Tissue procurement

As stated before, the low quantity of tissue available from human cancer has been one of the limiting factors for the application of proteomics to cancer research. However, the term ''low quantity'' depends first of all on the sensitivity of the technique used, and then on tumor type and tissue-procurement protocols.

For example, a sarcoma weighing several kg might provide several g of tissue for analysis, which would be enough even for the least sensitive 2D-PAGE analysis; however, a small needle biopsy will never provide enough ''leftover'' tissue for research purposes, unless extremely sensitive techniques are used. However, the same 5-kg sarcoma might be useless if not received following optimized procedures, starting from patient consent and ending in proper handling and storage.

2.1. Human tissues, primary cell cultures and cell lines

There are frozen cancer-tissue repositories in many pathology departments for diagnostic and research purposes. Most of these repositories started many years ago (as far back in time as the 1970s!) when molecular techniques started to be used in cancer diagnosis and