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A proteomics approach for the development of sarcoma biomarkers

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ABSTRACT

Sarcomas are rare and clinically diverse malignancies, and treatment optimization requires the development of suitable biomarkers. In earlier research employing proteomics analysis, we identified peroxiredoxin 2 as a biomarker of osteosarcoma (OS) that can predict response to neoadjuvant chemotherapy and verified its functional significance in the resistance of OS cells to chemotherapeutic drugs. In addition, in gastrointestinal stromal tumor (GIST), we identified pfetin as a prognostic biomarker and validated its prognostic utility in multi-institutional studies by immunohistochemistry. Here, we present an overview of our progress in sarcoma proteomics and discuss future perspectives.

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1. Clinical features of sarcomas and the need for biomarker development

Sarcomas are rare and highly diverse mesenchymal malignancies, arising from bone, cartilage, muscle, fat, peripheral nerves, and adipose or fibrous connective tissues [1]. Sarcomas affect ~200,000 individuals worldwide each year, accounting for less than 1% of all adult malignant tumors. Sarcomas are classified into more than 50 histological subtypes, many of which have unique clinical, prognostic, and therapeutic features. Clinically, sarcomas range from curable tumors to those causing aggressive, incurable disease. Standard chemotherapy protocols have been established for only few sarcoma types; for others, numerous molecular-targeting treatments are currently under investigation [2]. In order to optimize the response of sarcomas to therapeutic intervention and minimize any treatment-related toxicity that could compromise clinical efficacy, novel biomarkers are urgently required.

2. Proteomics approach toward characterization of biomarkers using tumor tissues

The proteome is a functional representation of the genome that directly characterizes cell or organism phenotypes. Proteomics can provide unique proteome data on the level of protein expression [3,4]; status of protein complexes [5,6]; and post-translational modifications such as phosphorylation, ubiquitination, glycosylation, acetylation, and ribosylation [7,8], protein localization [9,10], and protein function [11,12]. By integrating these data with clinical observations, it may be possible to identify biomarkers that could be useful for evaluating the malignant potential of different sarcomas.

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For studying the sarcoma proteome, we had employed two-dimensional difference gel electrophoresis (2D-DIGE) [13], where protein samples labeled with different spectrally resolvable fluorescent dyes are separated by 2D gel electrophoresis (2D-GE). The gels are then laser-scanned using dye-specific excitation wavelength enabling to see each sample separately. In general, 2D-DIGE allows more comprehensive protein expression profiling than classical 2D-GE, which is especially important for the studies of cancer biomarkers [14–16]. First, gel-to-gel variations can be compensated for by using a common internal pooled control (a mixture of individual samples labeled with a fluorescent dye different from that of individual samples) allowing quantitative comparison of multiple specimens. Second, 2D-DIGE provides parallel assessment of several experiments, which is essential for biomarker studies based on repeated examination of many samples within a relatively short period of time. In 2D-DIGE, it can be achieved by simultaneous use of electrophoretic instrumentation, since the cost of performing electrophoresis is relatively low. Moreover, protein detection is performed using laser scanning, allowing high-throughput analysis of multiple samples. Third, contrary to conventional 2D-GE, in 2D-DIGE large-format gels displaying thousands of protein spots can be used [17,18] because, contrary to colorimetric staining, the detection by laser scanning does not require handling of fragile, easily breakable polyacrylamide gels. In our experiments, up to 5000 protein spots could be observed in a single gel during 2D-DIGE [16]. Finally, 2D-DIGE offers high sensitivity of protein detection [19], which is critical for biomarker studies based on size-limited clinical specimens. We have reported that very small samples (micrograms of protein), such as those obtained by laser microdissection, can generate thousands of protein spots after labeling with a highly sensitive fluorescent dye [19] (a detailed protocol published in [16]). Thus, the major advantages of 2D-DIGE as a modality for proteomic biomarker studies are comprehensive high throughput analysis, high sensitivity, and reduction of inter-gel variability, which provides quantitative comparison of biological changes and reduces experimental bias. The combination of these factors makes 2D-DIGE a more reliable approach to the identification of clinically relevant cancer biomarkers for prediction of treatment response compared to other proteomic techniques, which may be superior in terms of individual parameters. That is why 2D-DIGE has been widely used in biomarker studies resulting in identification of a number of proteins that correlate with clinical parameters in various cancers.

3. General strategy for biomarker development

In 2002, Anderson et al. published a landmark paper where they evaluated proteins according to the expected expression levels in plasma [20]. At that time, conventional proteomics made limited contribution to the list of plasma biomarkers used in the hospitals. Since then, the advances in proteomic technology resulted in considerable improvement of the sensitivity in protein detection, holding promise that comprehensive proteomics might instantly lead to successful biomarker identification. However, global expression profiling alone may not necessarily be so successful. Thus, transcriptomics, which quantitatively monitors mRNA synthesis using DNA microarray technology, has been extensively used in biomarker studies over the last decade [21–23]. However, among hundreds of reported mRNA candidate biomarkers, only few advanced to clinical application [24,25], suggesting that although comprehensive technology is potentially beneficial for biomarker discovery, it may not warrant successful identification of clinically relevant molecules.

Biomarker studies should be based on profound understanding of disease background and conducted to meet clinical demands [26,27]. Based on specific clinical requirements, samples should be appropriately stratified according to clinical characteristics, and informative proteins can be revealed through comparative studies. However, researchers conducting basic proteomic studies generally do not have medical background or access to clinical data. Therefore, interdisciplinary collaboration between basic and clinical scientists is mandatory in biomarker studies.

On the basis of this concept, in 2004, we launched a collaborative project on sarcoma proteomics that united efforts of basic and clinical researches. Six young medical doctors specializing in sarcoma have participated in our sarcoma proteomics project. In Japan, basic laboratory experience is a mandatory part of medical training, and employing a problem-oriented research style, we have incorporated clinical approach into our proteomics project.

The biobanking system at the National Cancer Center in Tokyo provides an invaluable source of clinical samples for research purposes. Tumor tissues (frozen in vapor nitrogen) from over 1000 sarcoma patients are stored anonymously but could still be traced by medical records such as pathological diagnosis, treatment, and clinical outcome. These samples have been the basis of our sarcoma proteomics project.

In addition to the proteome, we also used the data on the sarcoma transcriptome and genome and found that most of this information does not overlap and that integration of these data is quite challenging. These issues will be discussed elsewhere.

4. Approach to study sarcoma proteome

When we began sarcoma proteomic studies in 2004, there had been no established approaches to proteomics in cancer research, especially with regard to sarcoma. When we employed 2D-DIGE to generate global protein expression profiles of 80 soft-tissue sarcoma samples with seven different histological backgrounds, we found that histologically identical sarcomas shared common proteomic features [28], consistent with the results of a previous DNA microarray study showing a similar tendency for the sarcoma transcriptome [29].

Sarcomas exhibit a clinically wide spectrum from curative to malignant disease, the latter being associated with metastasis and treatment resistance. These clinical characteristics often correspond to histological subclasses and grading [30]. Accordingly, our preliminary observations encouraged further proteomic studies as part of general efforts in the discovery

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