Fingerprinting in cancer diagnostics

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Cancer is 'a plague of the 21st century', and is a threat to humans. At the same time it presents a subject of investigation for the broader scientific community. Successful treatment of the disease depends on numerous factors such as prevention, and early and sensitive diagnostics. That means the sooner a cancer is detected, the better the chances are of treating it effectively [1,2]. Thus, it is not surprising that new methods, arrays and techniques are still being developed for this purpose.

History
In 1937, Rudolf Brdicka published his discoveries regarding the use of polarography to diagnose a tumor disease in *Nature* [3]. He discovered a sensitive polarographic 'protein effect' conspicuously exhibited by serum. This phenomenon was explained by the catalytic activity of the sulphydryl groups of proteins. The 'protein effect', represented by a characteristic wave on the current voltage curve, has always been found to be larger in normal serum than in cancer serum samples [3]. One year later, Brdicka's colleague Jaroslav Heyrovsky, Nobel Prize laureate in Chemistry 1959, published a paper in the same journal in which he summarized results obtained in the field of polarographic research on cancer [4]. Heyrovsky believed that this field of study would be of general interest to many scientific groups around the world, but this was not the case. Since then, electrochemistry has slowly been disappearing from tumor disease diagnostics while being replaced by modern analytical and molecular biology techniques. Thus, this unique and interesting method has not been widely used, with only a few exceptions [5], for more than 50 years.

Metallothioneins
These low molecular intracellular proteins, rich in cysteine, are able to bind heavy metals in their structure [6–9]. The generally accepted idea that metallothioneins (MTs) are involved only in storage, homeostasis and detoxification of metal ions is now changing owing to recent findings showing that they are involved in apoptosis inhibition, immunomodulation, regulating transcription, cell proliferation and enzyme activation via administration of zinc atoms to the proteins and regulation of their concentration. MT genes are regulated in a tissue and isoform-specific way by numerous factors, including a general responsiveness to zinc and other dietary factors, inflammation, environmental stress and cell proliferation, which is related to cancer. Moreover, the level of MT can be related to the efficacy of treatment with certain drugs, for example, cancer chemotherapeutic agents. Another field currently receiving considerable attention is the value of MTs as biomarkers of zinc status, metal exposure and the prognosis of certain cancers [10]. Moreover, there is some evidence that elevated heavy metal and MT content in tumor tissues is connected to the increased invasivity and metastasizing of a tumor [11]. Besides the understanding of the role of MTs, essential and nonessential metals in carcinogenesis and tumor

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growth, the investigation of metal distribution within a tumor can answer many important questions concerning the growth of the tumor and its regulation. Understanding this phenomenon can subsequently lead to the discovery of new approaches of tumor growth inhibition. For this purpose, a combination of various analytical approaches is needed.

Mathematical analysis

Besides electrochemistry, techniques such as immunochemistry, mass spectrometry and electrophoresis generate from tens to hundreds of signals somehow relating to the sample composition, but there is no complete model for the behavior of proteins. It is almost impossible to process all such data manually, even with the help of instrument software. It is probable that each proteome must be characterized, combining the information obtained by several independent analytical approaches. But finding such relational dependencies is out of the scope of manual evaluation of provided data as it leads to combinatorial explosion [12]. This task cannot be resolved without efficient computer-supported decision making based on the results of sophisticated preliminary data-mining analysis that will design novel means for representation and interpretation of the considered data.

> "It is obvious that mathematical treatment of analytical signals using sophisticated mathematical tools could be very beneficial not only for chemists, biochemists and biologists, but also for mathematicians."

Data mining has been studied and applied for nearly two decades. Numerous methods for data processing and modeling have been designed, tested and are now available commercially or as open source tools ready to support analysis of typical datasets. However, the analysis of scientific data still remains a big challenge, especially since each new task, with its own specific requirements and constraints, calls for the design of a targeted data preprocessing approach, for novel data representation and for modeling solutions that make it possible to take into account the domain knowledge as a guide during the search for a reasonable hypothesis. The more methods that exist, the more difficult the task of the data-mining expert, as the expert needs tools to quickly assess the properties of the provided data to reveal attribute interactions and the type of relations hidden in the data. Such information can help to choose the proper tools to be applied, identify interesting subsets, motivate enhancement of the considered data and point to some surprising properties of the considered data. Data visualization coupling human pattern recognition and problem-solving capabilities seems to offer an attractive assistance – this is why visual data mining has attracted a lot of attention recently. The challenge is to design and create clear, meaningful and integrated visualizations that support interaction between data

miners and data producers, who possess the domain knowledge that is necessary for identification of interesting directions for further data-mining activities.

Electrochemical fingerprinting

In our studies, we took advantage of the mathematical tools and electrochemical analysis of MT. Primarily, we aimed at *in vitro* interaction studies of interactions of MT with cisplatin. To evaluate the results, interaction constants were suggested. Here, we found that the maximum increased interaction occurred, when conservative amino acids were substituted for more than one position outside the cysteine cluster. On the contrary, amino acid substitution within the cysteine cluster led to a reduction in interaction constants. This result clearly indicates that amino acids outside the cysteine binding motif are of high importance for interactions of MT with anticancer drugs [13,14].

A mathematical approach was also utilized for evaluation and classification of datasets obtained by electrochemical determination of MT in tissues. Based on our results, we were able to construct a decision tree distinguishing among electrochemical analysis data resulting from measurements of all the considered tissues [15]. It is obvious that there have been many attempts to provide fingerprinting of a tumor disease, however, these attempts suffered from very complex matrices and large sets of unprocessed data. Recently we tested the potential of the visual data-mining approach on data provided by Brdicka's curves. Our intention has been to distinguish various biological samples from diverse resources. The obtained results are more than encouraging [16]. They indicate that fingerprints for some cancers could be based on the results of sophisticated mathematical transformation of Brdicka's signals.

> "There must be a good hypothesis, and then meaningfully used mathematical tools can possibly help find the so-called 'holy grail'."

It is obvious that mathematical treatment of analytical signals using sophisticated mathematical tools could be very beneficial not only for chemists, biochemists and biologists, but also for mathematicians. On the side of the producers of the data, there is the possibility to discover some at first sight unknown phenomena. However, producing lots of data without any idea only to 'hunt ghosts' in chromatograms, mass spectra, voltammograms and other types of analytical data representations is more or less useless. There must be a good hypothesis, and then meaningfully used mathematical tools can possibly help find the so-called 'holy grail'. Sophisticated mathematical tools can be also used to mimic biologically complex processes such as ribosomal protein synthesis [17].

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