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REVIEWS

Atomic-level cancer studies – a new challenge to distal interdisciplinary science

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Abstract

Background: Nowadays, the amount of cross disciplinary research in many branches of science is growing dynamically. Multidisciplinary, interdisciplinary and transdisciplinary evaluations are being carried out. Scientific progress, in combination with the development of knowledge and new technical discoveries, allows the most complex problems to be considered in an innovative way, which results in original solutions, not present in the previous, more traditional understanding.
Materials and methods: We reviewed the current data on the evaluation of pathological tissues using current mass spectrometry methods, including advanced isotope ratio mass spectrometry in the first known atomic level cancer studies.
Results: The first attempts to estimate the stable isotope ratio in pathological tissues appeared as a potentially new method of understanding the biology of cancer. The changes in stable isotope concentration showed the relationship with the fundamentals of cancer – its promotion stage, and the mechanisms of its progression as well as its relationship to the standard prognostic biomarkers. The advanced multidisciplinary analysis of the results of isotope ratio assessment in cancer studies culminated in the creation of 'The Revolve(r) Heavy Nitrogen Theory of Death', which explains the phenomena of the proliferation force of cancer cells.
Conclusions: Stable isotope profiling performed using the highly advanced and creditable isotope ratio mass spectrometry approach reveals the previously unknown area of pathological tissue biology. Overcoming interdisciplinary barriers opens a new branch of medical studies with a potential clinical application. The intriguing results of the first attempts and potential clinical implications should form the basis of the next atomic-level cancer studies projects.
Keywords: cancer, biomarkers, stable isotopes

Introduction

Advanced interdisciplinary approaches are located at the crossroads of the traditional methods of evaluation, and their rapid development is currently being observed [1]. These tools are also highly valued due to their extreme reliability, which fulfils even the highest demands; this has been proved in forensic medicine investigations [2]. The same reliability allows

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interdisciplinary projects in medicine with a potential clinical application to be conducted, which is especially necessary in cancer studies for the purposes of routine oncology.

The literature indicates that success in interdisciplinary projects depends on many factors, namely the selection of research leaders and scientists, motivation, communication and external support, as well as the availability of financial sources for the working groups [3–5]. Nowadays, there are funding programs devoted to promoting interdisciplinary research. The problems with conducting these studies appear to be both of a cognitive and epistemic nature, and those that affect the effectiveness of multidisciplinary research must be better understood [6]. Despite the many advantages, some mistakes characteristic of interdisciplinary studies have been observed, such as inappropriate selection of disciplines, aims of the studies, members of research teams, methods or indicators of the expected solutions, as well as environmental sources of problems that are difficult to avoid, e.g. language barriers, lack of experience or insufficient funds, and the necessity to create new methodological modes [5,7].

The relationships between branches chosen for interdisciplinary studies have to be evaluated by the scientist who wants to carry out such demanding projects. It is pointed out in the literature that there are important differences between close and distal types of interdisciplinarity in scientific research. The first results in projects that are easy to carry out as well as numerous benefits, whereas the second – distal interdisciplinarity (if the projects successfully reach their aims) – only yields a limited number of solutions [3–5].

Interdisciplinarity in medicine shows has great potential and also appears to be a source of new clinical, diagnostic and therapeutic protocols, or leads to the implementation of more comprehensive patient care [1,8–10]. Interdisciplinary research proves that combining areas that by their nature have little in common with each other allows hitherto unknown aspects of the phenomena under investigation to be revealed, including disease processes, which, as emphasized, is of particular importance for the further development of medicine [1]. Distal interdisciplinary findings in medicine are resulting in a new generation of biomarkers with a potential clinical application.

Distal interdisciplinarity in mass spectrometry cancer biomarkers

The National Institutes of Health Biomarkers Definitions Working Group (1998) reports that a biomarker is a trait that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. The

working definition of biomarkers is still evolving and new groups of these factors are being introduced and modified along with technical and scientific progress. At present, the most rapidly developing group appears to be molecular biomarkers – the genes themselves and their products, as well as changes at the mRNA level [11]. They are already being introduced into oncology and thanks to their documented clinical impact have made a real breakthrough in its routine procedures.

For the purposes of medicine, biomarkers, which can aid in clinical evaluation, are divided into two groups, namely prognostic and predictive parameters [11,12]. Optimal biomarkers are those that are highly specific and can be measured without difficulty in easily obtained materials, e.g. body fluids, such as saliva, blood serum and urine, or they are available with limited injury/complications, e.g. by fine needle aspiration biopsy. The prognostic factors emphasize the predicted natural course of the disease and the prognosis; they are also associated with overall survival (OS). Predictive parameters are related to the likelihood of a positive response to the treatment given. The search for new tumor biomarkers, as well as the achievement of optimal treatment results [12,13], remains the goal of creating new, effective therapeutic protocols.

Historically, the first breakthrough in prognostic parameters was made in immunohistochemistry. This traditional diagnostic method, used routinely in differential diagnosis, appeared to be of prognostic value; the relationship between expression levels was idenitifed and the prognostic parameters were documented [14]. Nowadays, the most numerous biomarkers are of a molecular nature, and they are widely identified via proteomic analysis. Thanks to the use of cancer biomarkers, many aspects related to cancer can be analyzed. The evaluation of these parameters allows one to predict the cancer growth (e.g. BRCA1 germline mutation in breast and ovarian cancer), conduct screening tests (e.g. PSA in prostate cancer), assess targeted therapy utility (e.g. KRAS and antibody anti-EGFR in colorectal cancer), and monitor the disease (recurrences by CEA estimation in colorectal cancer) [12,13,15].

The additional benefits of biomarker estimation is that they make it possible to shorten the treatment time and at the same time minimize both negative side effects and the costs of the therapy. Therefore, the main aim of many contemporary studies is to search for new ways to reveal new biomarkers, which are of special importance for cancer patients.

The dynamic development of mass spectrometry, for which four Nobel Prizes have been awarded and which has already opened a new area of biomarkers, shows, especially in proteomics, new directions for studies with the use of high-resolution mass spectrometry. Proteomics is a new discipline

that aims to investigate the proteome – the protein component encoded by the genome (PROTEin complement of the genome). The creation of a separate research unit (Human Proteome Organization) is to lead to the classification of proteins, similar to the cataloging of genes in the Human Genom Project. Until the 1970s, protein masses were determined using methods such as electrophoresis, ultracentrifugation and chromatography, but the measurement error was 10% to 100%, which precluded clinical studies of human tissues. Only the introduction of mass spectrometry in the nineties of the last century made it possible to study proteins while maintaining high sensitivity of measurements. The usefulness of the mass spectrometry method for the search for new biomarkers in pathological conditions, especially cancer, is currently well documented [16].

Mass spectrometry methods

Mass spectrometry (MS) is an analytical technique based on measuring the mass-to-ion charge ratio (m/z) in the gas phase. Mass spectrometry substances to be studied with the use of the mass spectra of atoms and their molecules. The essence of the method lies in the fact that ionized atoms or molecules of substances are separated by the value of the m/z ratio and recorded separately using a mass spectrometer. From the mass spectrum obtained, the mass values and the relative content of individual components in the sample are determined. MS came to the fore at the turn of the 19th and 20th centuries thanks to the research work of Joseph John Thomson and Francis William Aston. Joseph John Thomson, the discoverer of the electron who researched electrical conductivity in gases, was awarded the Nobel Prize in Physics in 1906. The research carried out by him and his student, Francis William Aston, resulted in the construction of a mass spectrometer. For his invention and research on isotopes, in 1922, Aston also received the Nobel Prize – in chemistry. A breakthrough discovery in spectrometry was its coupling with gas chromatography in 1956 (GC-MS, English: Gas Chromatography – Mass Spectrometry), which made it possible to separate the mixture and perform a qualitative analysis of all its components. The development of the method of electrospray ionization (ESI) has led to the development of another combined technique – liquid chromatography-mass spectrometry (LC-MS). The invention of the ion trap – a new type of analyzer, resulted in 1989 in another, and not the last, Nobel Prize in the history of mass spectrometry – in Physics, for Wolfgang Paul and Hans Georg Dehmelt. The fourth Nobel Prize, in the field of chemistry, was awarded to John Fenn and Koichi Tanaka in 2002 for the use of electrospray ionization in the analysis of biopolymers and the

development of a new research method – Matrix-Assisted Laser Desorption Ionization (MALDI), and its use in conjunction with a Time of Flight (TOF) analyzer. Contemporary MS is a highly developed technique of mass measurement. The devices allow molecular weight to be determined with previously unattainable accuracy, especially with the use of nuclear cyclotron resonance with Fourier transformation [17,18]. MS definitely has a competitive edge in comparison to other analytical methods, mainly due to the possibility of identifying substances present in very low concentrations, as well as the analysis of complex mixtures. The most commonly used methods of ionization are solid matrix laser desorption and electrospray, and the most valuable for tissue estimation is MALDI due to the possibility of assessing molecules directly on tissue sections without the need to homogenize the material [19–21].

Currently, cells, tissues and body fluids (mainly plasma) are being tested, which is yielding reliable results from samples with a very low concentration of the proteins being searched for (less than 5 femtomoles) [22]. MS may also be combined with protein databases and programs for de novo sequencing. In proteomic research, biochemical, physicochemical and bioinformatic methods are used, which enables the simultaneous analysis of thousands of proteins. New branches of this method have appeared e.g. interactomics (interactive proteomics) that studies the interactions between proteins at the atomic, molecular and cellular level. Although primary, proteomics has found application mainly in non-clinical areas. Currently, the leading direction in the development of proteomics is diagnostics, which confirms the existence of pathological changes, their differentiation, and determines the boundaries of pathological lesions, as well as clinical proteomics. This focuses on disclosing the differences between the proteome of healthy and sick people, leading to the disclosure of biomarkers of pathological processes (alleles of genes, metabolites or proteins) and to the search for changes in protein profiles caused by the therapy implemented [23–26]. It is assumed that the assessment of multicomponent sets of proteins and peptides selected from the complex proteomic profiles of blood serum will have a clinical application [27].

Proteomics is attempting to conquer modern oncology, its achievements have revolutionized the concept of the biomarker, which has been used so far and which is now defined as a measurable element that correlates with a normal phenotype, pathologically changed (including cancerous) or resulting from the therapy applied. The new term “cancer proteomics” has also been introduced, i.e. the creation of tumor protein profiles including proteins in tumor cells, proteins in the microenvironment and proteins appearing in the body fluids of patients with neoplastic disease [28,29].

Thanks to the use of LC-MS, LC-MS/MS, MALDI-MS/MS and MALDI-TOF-MS, it is possible to identify and analyze selected peptides and proteins in detail, as well as study endogenous compounds that occur in very low concentrations [30]. What is worth underlining is that nowadays it is possible to identify proteins characteristic of neoplastic diseases *in vivo* and to visualize them [31].

There are also attempts to use mass spectrometry as a method of molecular imaging of tumor tissues. In this technique (imaging MS), TOF spectrometers ionize proteins that are on the surface of cells or tissues, and then create mass spectra of the proteins in various positions. The protein profiles obtained are correlated with the microscopic image and related to modern histopathological classifications. The number of proteomic studies on tumors is rapidly increasing. They include the biology of cancers, such as lung, breast, prostate, colon, ovarian or endometrial cancer [32–37]. In addition, the correlations with recognized prognostic factors are analyzed [38].

There have been reports in the literature about the potential use of proteomics to distinguish primary and metastatic cancers. Using mass spectrometry, it is also possible to identify post-translational modifications that are considered by some researchers to be key in the development of cancer and the bioimage phospholipids of cell membranes [39–41]. Nowadays, the main goal of cancer proteomics is to identify diagnostic, prognostic and predictive markers [1] and fully personalize the treatment of patients [34,42,43].

Unfortunately, many valuable and extremely interesting achievements of proteomics have no clinical implications, as their use would require the creation of complete biomarker panels and to assign them to specific pathological conditions, while maintaining the expected sensitivity and specificity of the results obtained. Similarly, most of the other valuable findings in medicine are resulting in a lot of new markers, although their low specificity considerably limits direct clinical use [44,45].

Atomic level cancer studies

Atomic level cancer studies appears to be a unique example of distal interdisciplinarity with a potential clinical application. The studies are based on highly advanced techniques from the mass spectrometry panel, especially isotope ratio mass spectrometry, which relates to the atomic level of the evaluation of tumor biology, which has unexpectedly appeared to relate the isotopic composition of cancer tissue to cancer progression and dissemination with potential clinical implications. The results also brought a new type of isotopic biomarkers [10,46–52], as well as an innovatory idea for personalized cancer management via isotope fractionation

processes [52]. It should be mentioned that this method is also used for the evaluation of non-cancerous human pathologies e.g. diabetes mellitus or sepsis [8,9].

Isotope Ratio Mass Spectrometry (IRMS) makes it possible to reveal the distinctiveness of the materials tested by assessing their isotopic composition. This method measures the heavier isotope ratio to a lighter solid isotope of a given element, which makes it possible to see the phenomenon of isotopic enrichment or depletion (increase or decrease in the amount of a heavier isotope) in the test material treated as a whole. There are a number of contemporary applications for this unique technique with the highest reliability and precision of determinations that have been documented; it is growing rapidly. They include geographic identification and biological origin of the samples and environmental changes in the history of the Earth, identification of food sources, nutrient circulation, water assessment, metabolism research, doping control, which reveal the common origin of a substance e.g. drugs, explosives, identification of GMOs, and monitor man-made environmental hazards.

Changes in the isotopic ratio of elements are extremely small and, for ease of use, are expressed by the value delta (δ) expressed in per mille. A relative measurement is used, i.e. one which alternaes between the sample measured and the standard, within one measurement cycle, and relates the measured isotopic composition of the sample to the isotopic composition of the standard adopted. The delta value, therefore, expresses the relative difference between the isotope ratio in the sample and in the standard. Measurements of such high precision are made using an Isotope Ratio Mass Spectrometer, adapted to the study of the isotopic ratios of light elements – hydrogen, carbon, oxygen, sulfur and nitrogen [53].

Despite the many advantages and the highest credibility, only a few reports on the use of isotope mass spectrometry seem to concern studies with potential clinical impact. They evaluate the pathological processes in modern humans. They focus on the cognitive or traditional, non-medical aspects of IRMS measurements, and the evaluation covers only the non-invasive or minimally invasive samples, not post-surgical materials [8,9,54–57]. Subsequent publications are also starting to describe attempts to initially assess biological materials in the course of cancer, but they focus on the measurement of the isotopic composition of metals using methods based on inductively coupled plasma mass spectrometry. Furthermore, the concept of these studies, which are based on a small number of cases, does not include histoclinical analyses or the search for potential relationships with the spectrum of prognostic factors recognized in modern oncology, depending on the cancer type [46,47,51].

IRMS as a tool of Earth sciences appears to be barely accessible and very expensive for medical research, although the method meets the highest requirements concerning scientific value, especially in medicine for the purposes of evaluations with potential clinical application [2].

In 2015, structured atomic level studies were shown to be applicable to the direct evaluation of cancer tissues growing in vivo [7,8], and in the same year, for the first time, the statistical significance of such estimations and their prognostic value was proved [52], highlighting the last previously undiscovered area of cancer biology.

The prognostic impact of atomic cancer tissue evaluation was confirmed in 2016 in two independent studies on Wilms tumor [10] and breast cancer [50]. Although recently discovered, the utility of IRMS studies for medical purposes and their clinical application have currently made this method the standard tool in the new rapidly developing field of atomic level studies of pathological processes.

Summary

The interdisciplinarity of atomic level studies of pathological tissues draws attention to previously unknown aspects of the biology of abnormal tissues. Primary attempts to estimate the stable isotope ratio in pathological tissues appear to be a potentially new method of understanding the biology of cancer. The changes of delta values (isotope depletion or enrichment) show the relationship with the fundamentals of cancer e.g. the promotion stage of neoplastic disease, and the mechanisms of its progression as well as the established prognostic biomarkers of the cancer types examined.

Isotope mass spectrometry cannot be perceived as a competitive technique in relation to the recognized research method, which is the microscopic evaluation of cancer tissue, but it seems to be a valuable supplement to it. A stable isotope ratio in tumor tissue appears to be a novel isotopic biomarker of cancer. Finally, an advanced multidisciplinary analysis of the results of isotope ratio assesment in cancer studies has brought unexpected conclusions and resulted in 'The Revolve(r) Heavy Nitrogen Theory of Death' being written, which explains the phenomena of the proliferation force of cancer cells [58].

Conclusions

Stable isotope profiling performed with the use of the highly advanced and credible isotope ratio mass spectrometry approach reveals a previously unknown area of pathological tissue biology. Overcoming interdisciplinary barriers opens a new branch of medical studies with a potential clinical

impact. The intriguing results of the first attempts and potential clinical implications should also be the focus of the next atomic level cancer studies projects.

The author declares no conflict of interests.

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