International Journal of Applied Sciences: Current and Future Research Trends
(IJASCFRT)
ISSN (Print) , ISSN (Online)

https://ijascfrtjournal.isrra.org/index.php/Applied_Sciences_Journal

Search for New Methods for Cancer Diagnosis

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Abstract

Numerous cellular components are being studied to identify new possibilities for effective cancer diagnosis. Researchers have proposed proteins, miRNA, micronuclei, DNA, and other biochemical or cytological objects as potential markers and targets for disease detection and management. Often quantitative changes of these objects, increase or decrease, are observed in the blood serum of patients, which correlates with the severity of the disease or the effectiveness of treatment. In this regard, today, as never before, the issue of cancer management is acute and the study of cellular proteins is highly appropriate and effective. Management of cellular processes is associated with the activation and inhibition of various metabolic pathways. Typically, manipulating a single protein causes a chain initiation of multiple reactions. It is therefore difficult to say unequivocally that quantitative modification, activation or inhibition of this or that protein may be an unconditional marker or target to solve the above-mentioned problem. It is common for proteins already recognized as markers to manifest themselves differently in individuals. We therefore believe that the study of diagnostic agents should be conducted in a complex manner parallel to the metabolic pathways associated with corresponding proteins. We investigated changes of ubiquitin levels in blood serum of cancer patients and healthy volunteers. Elisa test and subsequent statistical analysis were used for assessment. The results indicate the necessity of further investigation of ubiquitin and other proteins that may react or indirectly affect ubiquitin level variability.

Keywords*:* cancer; ubiquitin; diagnostics.

1. Introduction

The expediency of quantitative changes in the regulatory protein ubiquitin has been suggested by a number of scientists to explore new ways of cancer diagnosis. Ubiquitin is involved in the management of virtually every cellular process, both directly and indirectly, due to its diverse functions.

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The structural conservatism of ubiquitin warrants it to participate in protein trafficking, degradation, and synthesis, as well as in stimulating and inhibiting protein functions. These functions are performed with or without the involvement of the ubiquitin-26 proteasome multicomponent systems [1]. Ubiquitin is covalently conjugated to cellular proteins in the cytoplasm and nucleus of eukaryotic cells by the conjugating enzymes . In this process, several ubiquitin monomers are usually sequentially linked to each other. The multi-ubiquitin chain acts as a signal for degradation of target proteins in the 26S proteasome. Proteolysis by ubiquitin regulates various processes in the cell, including stress response, cell cycle, gene expression, and apoptosis [2]. Ubiquitin is involved in the pathogenesis of various diseases. Increase in extracellular ubiquitin levels have been reported in patients with neurodegenerative, muscular, cerebral and cardiac ischemic, cancerous, rheumatoid, and other diseases. Cell ubiquitin has been suggested to be involved in the production of amyloid inclusions and the regulation of hematopoiesis [3]. In recent decades, numerous studies have been devoted to elucidation of the molecular mechanisms involved in metabolism in the cancer reprogramming process [4]. Ubiquitination and deubiquitination are actively considered as modulators of cancer metabolism [5]. Unregulated ubiquitination and deubiquitination play an important role in reprogramming signaling pathways of tumor cell by modulating transcription factors and enzymes, like ligases and DUBs (deubiquitinating enzymes), involved in metabolism [Fig. 1]. Therefore, ubiquitin balance in cancer metabolism requires more research [6-8].

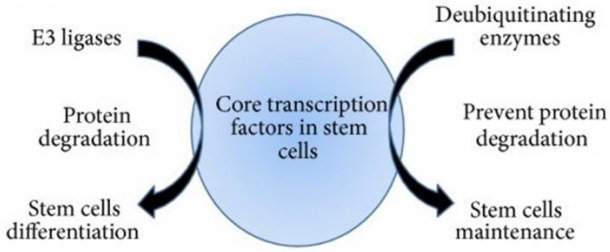
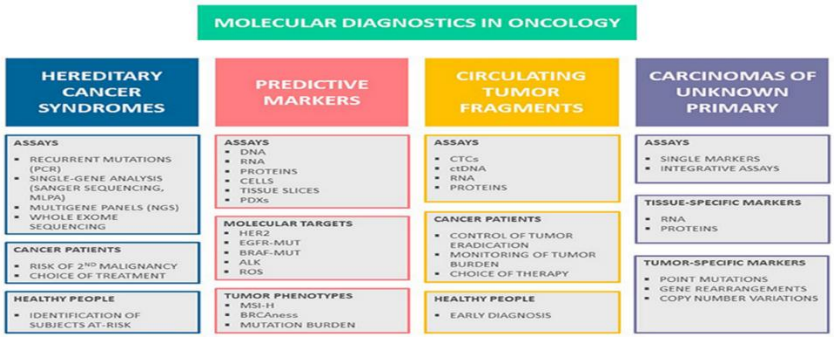


Figure 1: Schematic representation of the roles of E3 ligases and DUBs in regulating stem cell differentiation and stem cell maintenance.

Researchers have proposed proteins, miRNA, micronuclei, DNA, and other biochemical or cytological objects as potential markers and targets for disease detection and management [Tab. 1]. Often quantitative changes of these objects, increase or decrease, are observed in the blood serum of patients, which correlates with the severity of the disease or the effectiveness of treatment. Vast amount of proteins and peptides is considered as potential prognostic biomarkers to identify new possibilities for effective diagnosis [9].

Table 1: Front. Mol. Biosci., 27 August 2018 | https://doi.org/10.3389/fmolb.2018.00076



Main goals of cancer research are:

- To elucidate the biochemical and genetic mechanisms that underlie the uncontrolled growth of cancer cells
- To elucidate the biochemical and genetic mechanisms that underlie ability of cancer cells to invade and metastasize
- To develop successful treatments that destroy cancer cells, while causing minimal damage to normal cells.
- To identify protein markers for diagnostics and assessment of effectiveness of treatment.

In the search for effective diagnosis, the study of biologically active proteins is intensive. An innovative approach in this process is to explore proteins for which ubiquitin is a very favorable object because of its key characteristics. Ubiquitin is a natural serum protein with a conservative structure in the mammalian class and possesses high thermal, structural, and proteolytic stability. We suggest determining the importance of calculating cellular ubiquitin levels for the diagnosis of cancer and the ability to predict fluctuations in cell ubiquitin levels detected in the plasma of cancer patients [10].

2. Materials and Methods

Blood collection and serum preparation Blood was collected for conducting regular blood tests. The serum was prepared 10 minutes after taking the blood in gel-clot test tubes at room temperature. Then the serum was transferred to the Ependorf test tubes and stored at -8 degrees.

Elisa test Serum ubiquitin levels have been measured by Elisa test. Human Ubiquitin Elisa kit provided from LifeSpan Bioscience Inc. is a 96-well enzyme-linked immunosorbent assay for the quantitative detection of Human Ubiquitin in samples of Serum. Procedures were conducted according to the protocol proposed by the corporation. Absorbance on a plate was read at 450 nm wavelength, dilution 1: 100.

Statistical analysis Statistical methods OriginPro, ImageJ and ANOVA were used for quantitative analysis. Grouping of patients was made according diagnosis and control healthy group of volunteers. Research involving human participants performed in accordance with the requirements of the Council of Europe Convention on Human Rights and Biomedicine, Biomedical Research, as well as the UNESCO Declaration of

Bioethics and Human Rights and regulations established by the New Vision University Hospital ethic committee (Protocol N07/05.06.2017). We have obtained consent letters from volunteers to use their biological material for research.

3. Results

We aimed to study the quantitative changes of ubiquitin in the blood serum of patients with various diagnoses in order to show its diagnostic potential. In collaboration with several clinics, patients' blood serum was collected in the first phase of the study. The study was conducted using immunological methods. 70 patients and healthy volunteers were examined. Elisa quantitative test results and subsequent statistical analysis of the results of the immunological study showed quantitative differences in ubiquitin levels for divergent tumor diagnoses. The amount of extracellular ubiquitin varies widely according to the diagnosis. The results are as follows: Ovarian adenocarcinoma 13,6±6,2 ng/ml, Breast invasive carcinoma 33,6±8,9 ng/ml, Gastric adenocarcinoma 13,4±4,6 ng/ml, Intestinal gastric, rectal adenocarcinomas 25,8±4 ng/ml, Lung carcinoma 54±13,2 ng/ml, Cervical squamous cell carcinoma 37,9±8,6 ng/ml, Cervical adenocarcinoma 94±4.0 ng/ml. Significant differences in the serum ubiquitin levels of patients with different diagnoses compared with data from a control group of healthy volunteers - 31.7±3.9 ng/ml, indicate that it is not yet possible to discuss quantitative changes in ubiquitin for diagnosis [Tab. 2].

Table 2: Comparison of ubiquitin concentration in blood serum of patients with various diagnosis.

N	Cancer Type	Ub ng/ml
1	Ovarian adenocarcinoma 4 stage	13,6±6,2
2	Breast invasive carcinoma 4 stage	33,6±8,9
3	Gastric adenicarcinoma 3 stage	13,4±4,6
4	Intestinal gastric and rectal adenocarcinoma	25,8±4,5
5	Lung carcinoma	54±13,2
6	Cervical squamous cell carcinoma	37,9±8,6
7	Cervical adenocarcinoma	94±4
8	Other	41,4±8,5
9	**healthy**	31.73±3.9

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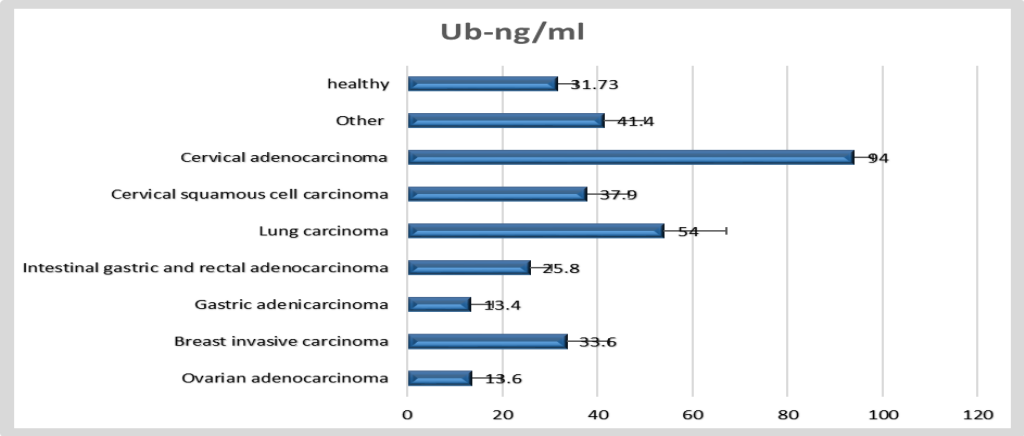

Figure 2: Study the quantitative changes of ubiquitin in the blood serum of patients with various diagnoses in order to show its diagnostic potential.

4. Discussion

The source of extracellular ubiquitin may be the passive release from cells during physiological processes such as apoptosis and necrosis, but some authors note the ubiquitin extraction from normal cells. To date, many aspects of extracellular ubiquitin activity remain unclear, spatially as to its possible pathways and role in the various cellular processes associated with malignant tumors. Cellular ubiquitin is considered a biomarker of the disease because many diseases are associated with increased ubiquitin concentrations in body fluids [11].

Ubiquitin activity is observed to be dysregulated in various kinds of cancers. Ubiquitination and deubiquitination can be abnormally regulated by transcriptional, translational, posttranslational or epigenetic mechanisms in cancer cells, exerting oncogenic or anticancer roles in carcinogenesis. The process of ubiquitin system participation in cancer progression, metastasis and probability of ubiquitin use for diagnostics requires thorough research [4, 12]. Management of cellular processes is carried out through the activation and inhibition of various metabolic pathways. Typically, manipulating a single protein causes a chain initiation of multiple reactions. It is therefore difficult to say unequivocally that quantitative modification, activation or inhibition of this or that protein may be an unconditional marker or target to solve the above-mentioned problem. It is common for proteins already recognized as markers to manifest themselves differently in individuals. We therefore believe that the study of proteins should be conducted in a complex manner parallel to the metabolic pathways associated with these proteins.

5. Conclusions

In recent years, the involvement of ubiquitination and deubiquitination within the regulation of metabolic reprogramming in cancer cells has received a growing attention. Given the complexity and importance of both cancer metabolism and protein ubiquitination, the precise roles of protein ubiquitin in metabolic reprogramming is worth further studies and analyses. The issue requires further investigation to determine the reasons for the sharp increase or decrease in protein relative to the norm and what accompanying metabolic processes should be considered for the effectiveness of the diagnosis. Today, as never before, the issue of cancer management is

acute. In this regard, the study of cellular proteins is highly appropriate and effective. More than one protein for identification of some types of tumors are necessary, otherwise diagnostics can be complicated and misinterpreted.

Acknowledgments

Society of Rheology, 405133029; Popularization of Rheology Science Program (PRSP); Project "Georgian reality: The sustainability of scientific research during the Covid-19 pandemic"

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