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Review Article

Unveiling Cancer Proteoforms: Insights into Biomarker Discovery and Therapeutic Strategies

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Key words
Biomarker discovery, Cancer biomarkers, Proteomics, Therapeutic strategies

ABSTRACT

Cancer proteoforms are an innovation in identifying and understanding biomarkers and reaching for effective therapeutic targets. Proteoforms can be defined as individual species with different functions and been derived from a single gene using post-translational modifications and other mechanisms including splicing. Unlike traditional fixed protein isoforms, proteoforms describe the dynamic and functional aspect of proteins, which are of great importance in cancer research and clinical treatment. The discovery of specific diagnostic and prognostic cancer biomarkers by proteomic techniques has transformed the prevailing practices in oncology. The investigation of cancer proteoforms has shown that these structural modifications of proteins can contribute to the cancer development, metastasis and therapy. Proteoforms identification and discovery at present employ sophisticated proteomic instruments and high-definition mass spectrometers. They enable researches to select and describe proteoforms with a high level of specificity however, they are in difficulties with the issues of standardization and validation. The incorporation of proteoforms information into genomics and transcriptomes will in turn improve knowledge about cancer and provide input about biomarker advancements. In the therapeutic applications, proteoforms targeted approaches are appear as novel strategies. There are cognitive strategies where drugs and therapies got developed to interact with targeted proteoforms, which can be possibly helpful to overcome resistance issues and enhancing the necessities of treatment. Many of these advances are held to be creating a new generation of cancer treatments tailored to bring about a change in the manner of handling the disease.

INTRODUCTION

Proteomics is the study of the proteome. The proteome encompasses the entire set of proteoforms present at a certain time in a cell, tissue, or individual in a given biological setting (Carbonara *et al.*, 2021). Proteomics includes the assessment of global protein abundance, proteoforms levels, spatial conformations, chemical modifications, cellular localization, proteoforms functions, cofactors, and interacting partner networks (Meissner *et al.*, 2022). The dynamic shift in the research of cancer

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has resulted in a consideration of proteoforms which are multiple isotypes of a protein that are produced from the same gene through the action of different biological mechanisms (Naryzhny and Legina, 2019). Proteoforms involve practically all the modifications, which can occur after translation or during transcription and can be seen as an exciting concept giving more dynamism to connections between protein structure and function in addition to isoforms (Uversky, 2016). It is essential to consider the proteoforms as a key molecular player in the case of cancer and design new generation diagnostics and therapeutic approaches (Su *et al*., 2024).

For this reason, proteoforms are different from protein isoforms since they are able to represent the complicated regulatory processes that occur in cells (Aebersold *et al*., 2018). With regards to cancer, then it can be observed that tumor cells have a distinct proteoforms variance compared to normal cells (Aebersold *et al*., 2018). This is an advantage since it offers the opportunity to investigate the tumor biology in addition to helping identify new biomarkers for the diagnosis, prediction of likely outcomes, and therapeutic targets (Mäbert *et al*., 2014). Certain prototypes linked to certain forms of cancer can act as biomarkers and provide a better picture of disease processes and tools for diagnostics and prognosis (Wu and Qu, 2015).

Proteoforms are not limited to discovery of biomarkers, they also provides the future possibilities for new therapeutic approaches (Su *et al*., 2024). Since several cancer-associated proteoforms are synthetically lethal or significantly contribute to resistance mechanisms, scientists can design corresponding molecular inhibitors to disrupt these disease-promoting proteoforms selectively (Gillette and Hill, 2015). This kind of approach is to maximize the treatment outcomes and minimize the side effects at the same time, and thus adhere to the concept of personalized medicine.

But the investigation of cancer proteoforms has its difficulties. Due to a high degree of proteoforms heterogeneity, conventional analytical approaches (Labib and Kelley, 2020) are insufficient to adequately identify proteoforms and their properties therefore, high-resolution mass spectrometry can be employed (van Schaick *et al*., 2022). Furthermore, proteoforms data need to be connected with other omics methods including genomics and transcriptomics for the better understanding of cancer processes (Zhang and Kuster, 2019).

With further development of the field, the diversification of cancer proteoforms can be considered a potent area of improvement for the diagnosis and treatment of cancer (Macklin *et al*., 2020). Thus, it is possible to expand the knowledge of proteoforms regulation to make further steps in constructing more effective and targeted treatments, which directly affects patient's health (Su *et al*., 2024).

UNDERSTANDING CANCER PROTEOFORMS

Cancer proteoforms identification show a great progress in the understanding and combating cancer (Macklin *et al*., 2020). A proteoforms can be defined as a proteins isoform that is produced from a single gene utilizing different biological processes such as post translational modification, alternative splicing and proteolytic cleaving (Aebersold *et al*., 2018). Such differences are important for understanding of the mechanisms of cancer and for creation of the diagnostic and therapeutic tools as shown in Figure 1 (Bertoli *et al*., 2015). This section brings more detailed description of the cancer proteoforms their definition, their implication in the cancer and the difficulties that are associated with the analysis of the proteoforms (Aebersold *et al*., 2018).

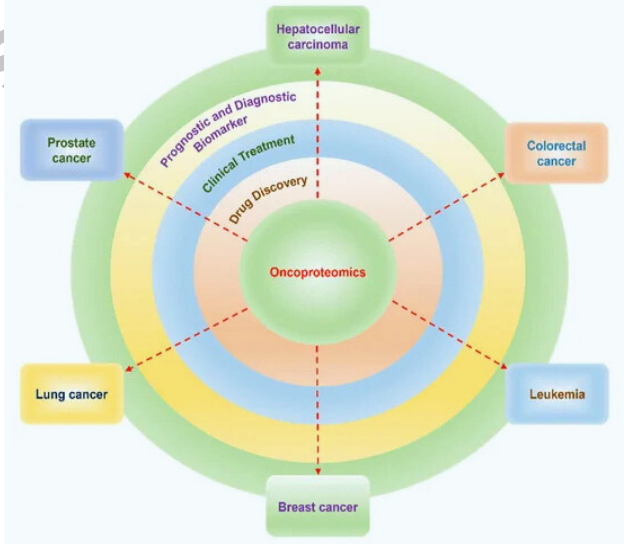

Fig. 1. Overview of oncoproteomics highlighting its role in biomarker discovery, clinical treatment, and drug development across major cancer types.

Role of proteomics in the prognosis and diagnosis of cancer

In cancer, cells show quantitative and qualitative differences in proteoforms composition as compared to normal cells (Ercan *et al*., 2023). These abnormalities can become altered expression of a protein, different modification of the protein or appearance of completely different protein fragment due to proteolysis (De Strooper,

2010). Understanding these variations is essential for several reasons.

Tumor progression

Proteoforms can affect process that are important for example, cell division, migration, and invasion (Naryzhny and Legina, 2019). For instance, wrong folding of proteins due to errors in glycosylation leads to different cellular adhesion which is associated with metastasis (Lin and Lubman, 2023).

Drug resistance

It is the variations with Proteoforms that may develop a resistance to the conventional therapies (Zhan and Su, 2024). Substances that cause changes in the effectiveness of treatment may include proteoforms that belong to certain enzymes in drug metabolism or signaling pathways (Su *et al*., 2024).

Immune evasion

It is proposed that some proteoforms modify surface antigen or secrete immune-modulatory factor, thus enabling the tumor to escape immune system (He *et al*., 2024).

ROLE OF PROTEOFORMS IN CANCER PROGRESSION AND HETEROGENEITY

Cancer is a disease that has been associated with several proteins and in this case, proteoforms have been established to play a significant role in this disease (Uversky, 2016). For instance, studies have bring into focus the fact that the existence of certain proteoforms of the prostate-specific antigen (PSA) enhances the ability to diagnose prostate cancer (Ferraro *et al*., 2023). Likewise, other altered proteoforms of metalloproteinase (MMPs) are associated with breast cancer development and metastasis (Cufaro *et al*., 2019). These examples show how knowledge of proteoforms can be useful in understanding the functions of malignant neoplasms and apply to the creation of more particular diagnostic tests and treatment strategies (Macklin *et al*., 2020).

Cancer proteoforms are different molecular structures of proteins which are produced from a singular gene via distinct biological procedures (Macklin *et al*., 2020). It is important for clinicians and researchers to learn about cancer proteoforms in order to compel some biomarkers for certain types of cancer as well as to initiate treatment processes (Lisitsa *et al*., 2014). This elucidates the cancer specific proteoforms resulted out of post-translational modifications (PTM), alternative splicing (AS), and proteolytic cleavage (PC) and discourses regarding the utility of these identified proteoforms in the identification and eradication of cancer.

Post-translational modifications (PTMs)

The PTMs are co-translational modification that relates to modification that happens to the proteins after the translation process (Raju, 2019). They refer them as functionally, spatially and structurally altering a protein to a very great extent. Several types of PTMs are commonly observed in cancer proteoforms. Several PTMs are found to be significantly associated with cancer proteoforms and the following are some types of PTMs noted:

Phosphorylation

This modification involves the formation of covalent link between phosphate group and the amino acid residues which can be any one of the three residues including serine, threonine and tyrosine (Hunter, 2012). Phosphorylation is associated with the processes that are relevant as regard to proteins and it also refers to the pathways, which can result in cancer development (Ardito *et al*., 2017). This kind of disturbance is observed in several diseases for instance breast cancer, prostate cancer among others.

Glycosylation

Of all the post-translational modification of proteins, carbohydrate addition to proteins has been referred to as glycosylation which affects protein folding, stability and cell-surface interaction (Walsh and Jefferis, 2006). Glycosylation is reported to be affected in a number of cancer types comprising ovarian and pancreatic carcinomas and the alterations help tumor cells to spread and evade immunity (Rodrigues *et al*., 2018).

Ubiquitination

This involves the labeling of the proteins using a molecule called ubiquitin, this results into degradation of the protein (Hershko and Ciechanover, 1986). We also confirmed that defective ubiquitin system causes the enhanced protein degradation and signaling pathways which are associated with cancers (Burger and Seth, 2004).

Alternative splicing

Another interesting mechanism of gene expression regulation is this phenomenon called alternative splicing in which different groups of exons are joined to form different proteins from the same gene (Gehring and Roignant, 2021). In cancer, alternative splicing can generate proteoforms with altered functions (Montero-Calle *et al*., 2023). In cancer, AS can produce proteoforms which possesses either different function or even gain new properties:

Exon skipping

This type of an alternative splicing implies that some of the exons are skipped hence may produce low molecular weight or non-functional protein products (Birzele *et al*., 2008). For instance, melanoma exon-skipping events lead to the formation of other proteins as biomarkers (Chen *et al*., 2024).

Intron retention

Due to presence of introns in mature mRNA, the proteoforms with the different functional capabilities are created (Aviña-Padilla *et al*., 2021). From a scientific perspective, intron retention has been related to the manufacture of proteins that install carcinoma and therapy resistances in cancers including glioblastoma.

Proteolytic cleavage

The others process is proteolysis, this is the enzymatic cleavage of proteins to smaller sections as shown in Figure 2. This process can generate proteoforms with distinct functions. This process can produce proteoforms with different activity levels (Aebersold *et al*., 2018), such as activation of proenzymes: Certain of the proenzymes are synthesized in compact form without an active core that has to be activated in order to become a functional molecule (Khan and James, 1998). In cancer, in the squamous cell carcinoma, activities such as the MMPs proenzymes which enhances tumor invasion and metastasis (Pornchai *et al*., 2001).

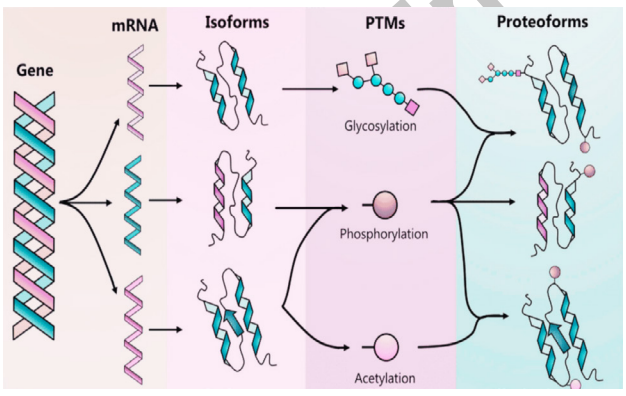

Fig. 2. Proteoform diversity arises from gene transcription, alternative splicing into isoforms, and post-translational modifications such as glycosylation, phosphorylation and acetylation.

Oncofetal proteoforms

Some of these proteoforms are overexpressed in cancer tissues and are in fact classified as Oncofetal proteins because of their up regulation during fetal development (Vainshelbaum *et al*., 2022). These proteins can serve as biomarkers for certain cancers. These proteins can be used as biomarkers of some types of cancer (Bhatt *et al*., 2010).

Alpha-fetoprotein (AFP)

AFP is a confirmed Oncofetal protein which is categorically overexpressed in hepatocellular carcinoma and in diseases associated with germ cell (Sideras *et al*., 2015). It has been ascertained that in diagnosing and staging of these cancers, serum AFP is useful because its concentration is high within the blood.

Carcinoembryonic antigen (CEA)

The other Oncofetal protein known to have linkages with different types of cancer including the colorectal cancer is the CEA (Goldstein and Mitchell, 2005). Perhaps, the concentration of this cytokine in serum can be used for the evaluation of therapeutic outcomes and the existence of the disease relapse (Carrieri *et al*., 1998).

ROLE OF CANCER PROTEOFORMS IN BIOMARKER DISCOVERY

Proteoforms which are molecular variants of cancer proteins resulting from genetic and post translational modifications, are so vital in biomarker discovery and utility for early detection, diagnosis and prognostic marker of oncological diseases (Shukla, 2017). Recent molecular markers are very selective in detecting cancer cells among normal cells and one cancer type from another (Maruvada *et al*., 2005). Different forms of PSA are more effective in diagnosing the prostate cancer than the total levels of PSA. In the same way, changes in glycosylation of the CA-125 proteoforms increases specificity as a biomarker that is useful in diagnosing and monitoring ovarian cancer (Schuster-Little *et al*., 2020).

PROTEOFORMS AND DISEASE PROGNOSIS

Proteoforms are rich in prognostic data since they indicate tumor malignancy and patient outcomes. There are Matrix metalloproteinase (MMPs) the variant being associated with cancer metastasis and short survival and in patients with increased MMP-2 and MMP-9 levels, the tumor is likely to be more invasive (Zhu *et al*., 2023). Aberrant splicing isoforms in breast cancer confers resistance to therapy and prognosis of patient's survival proteoforms variants assist with treatment (Qi *et al*., 2020).

Also, proteoforms are involved in assessment of treatment efficacy and identification of cancer relapses (Metwali and Pennington, 2024). Whereas lack of change

in certain proteoforms suggests poor responses, increased or decrease in particular proteoforms is a better indication of patient response to treatment (Brown *et al*., 2020). Tumor-specific proteoforms in biological fluids are useful in early detection of recurrence enabling early intervention and therefore better prognosis (Piana *et al*., 2024).

TARGETING PROTEOFORMS FOR PRECISION THERAPY

Proteoforms present therapeutic agents that potential uniquely on targeting the changed molecular characteristic of carcinomas (Su *et al*., 2024). Small molecules inhibitors can be rationally designed to selectively hit any pathogenic proteoforms. For instance, inhibitors selectively bind to certain proteoforms variants of kinases or other signaling proteins and impede cancer cell signaling and growth (Malaney *et al*., 2017). Cancer vaccine can be designed to target specific proteoforms that are attained by the cancer cells. These vaccines intend to develop an immune response against the specific proteoforms related antigens, so as to enhance the immunological recognition of the cancer cells (Fusciello *et al*., 2019). Investigations about proteoforms specific antigens may provide the key for developing highly effective therapeutic vaccines.

ADVANCED PROTEOMICS TECHNOLOGIES

Scientific developments in the past years have further improved the identification, quantification, and characterization of cancer proteoforms, opening the field to new horizons and application in cancer studies (Piana *et al*., 2024). Consequently, high-resolution mass spectrometry offers the most feasible and powerful method to analyze proteoforms (Carbonara *et al*., 2021). Modernization in MS technologies include the trends like ultra-high-resolution mass spectrometers instrumentations that helped in better elucidation of proteoforms variants even in cases of subtle post-translational changes. These advancements enable the top-down proteome of 10 protein samples and enhanced definition of new cancer-specific proteoforms (Ntai *et al*., 2016). Methods as label-free quantification and stable isotope labeling helped to analyze the alterations of the proteoforms abundance during different cancer stages and treatment (Li and Zhan, 2021). These quantitative methods offer information on dynamics and functions of proteoforms in connection with carcinogenesis as well as cancer treatment (Macklin *et al*., 2020). Next-generation single-cell proteomics tools are developed to investigate proteoforms on the single-cell level (Petrosius and Schoof, 2023). This approach is particularly useful to comprehend tumor heterogeneity and the proteoforms of a single cell in a complex and diverse tumor micro-environment. Single cell proteomics can help understand the role of different cell subtypes in cancer development and its treatment (Zhu *et al*., 2023).

INTERACTION WITH GENOMICS AND TRANSCRIPTOMIC

The combination of proteomics with genomics and transcriptomic provides a systems-level understanding of the impact on proteoforms variation and function in cancer (Karimi *et al*., 2022). Integration of these multi-omics methodologies unveils how genetic changes, specific splicing, and regulation impact proteoforms production and function and how it supports the creation of multi-targeted therapies (Ruffinatti *et al*., 2023). Proteogenomics strengthens this concept by integrating proteomic data with genomic and transcriptomic data to provide better identification of new proteoforms and their respective genes for providing a better understanding of molecular event in cancer (Kumar *et al*., 2016).

EMERGING THERAPEUTIC STRATEGIES

New therapeutic approaches that are being derived from the concept of cancer proteoforms will seek to take advantage of these proteins (Su *et al*., 2024). That is why one of the most promising strategies under investigation is the creation of drugs selectively acting on cancer-related proteoforms (Su *et al*., 2021). With the utilization of these proteoforms specific inhibitors and antibodies, they dynamically selectively the abnormally translated proteoforms as to impair its function in preventing tumor growth, and thus enhance therapy selectivity and reduce side effects (Forgrave *et al*., 2022). Furthermore, therapeutic vaccines which are based on proteoforms specific antigens and gene editing approaches including CRISPR/Cas9 (Mijakovac *et al*., 2022). These methods are the improvement of immune response to proteoforms which are unique to the tumor, as well as the elimination of mutations which lead to abnormal proteoforms production (Uversky, 2016). These have been powerful strategies that have large potential for improvement of cancer care.

CONCLUSION

Proteoforms, derived from genetic and post-translational alterations in cancer-associated proteins, provide intricate informative features about tumor biology, dynamics, and therapeutic prospects of heterogeneity and resistance. Recent molecular diagnostic techniques such

as high-resolution mass spectrometry and single-cell proteomics are identifying innovative cancer-associated proteoforms that are revolutionizing cancer diagnostics and therapy. There are limitations like proteoforms complexity, integration of datasets, and biomarker confirmation and they have to be solved to bring the findings into clinical utility. Future development will be also based on technological improvement, interdisciplinary collaboration and elimination of restrictions and moral issues. Finally, the elucidation of cancer proteoforms can further improve cancer individualization as well as provide profound advancement in the field of oncology.

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