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Review Paper

Emerging Biomarkers for Cancer Diagnosis: Paving the Way for Precision Oncology

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Abstract

Cancer is one of the most ubiquitously present diseases in the world along with cardiovascular disorders and diabetes. Decades have passed, but still the ultimate treatment for this disorder is not found. However, recent innovations in technology have developed some promising methods for the detection and treatment of this disease, therefore, giving rise to the field of precision oncology. Biomarkers are advanced methods used in the diagnosis and treatment of diseases, including cancer. Multiple bioassays have been developed for the diagnosis and prognosis of cancer including, circulating tumour cells (CTCs), liquid biopsy, exosomes, non-coding RNAs (ncRNAs), metabolomic markers and imaging biomarkers. All of these methods are found to be effective in malignant detection, progression and treatment. From the detection of circulating cancer cells, DNA, RNA and exosomes in blood to the imaging of the cancer metabolites in body, advancements have been made. Although these biomarkers are capable of treating cancer but various drawbacks are still present in their sensitivity and stability. In order to meet this issue, the combination of these biomarkers is recommended and found to be efficacious, such as, the integration of metabolomics and imaging techniques is found to be promising technique for precision oncology and targeted therapies against cancer. In this article, we will discuss the emerging biomarkers of cancer and how these bioassays are making the way for personalized medicine, precision oncology and targeted therapies.

Introduction

Cancer is a common term used for a group of disorders marked by random and uncontrolled cell division. It is one of the chief factors that causes mortality with a multifaceted disease physiology. Many external factors have been reported to induce cancer including improper diet, consumption of tobacco, unhygienic and morbid lifestyle and absence of physical activity (Quazi, 2022). Apart from them, mutation of pro-cancer genes, expression configuration of tumour suppressor genes as well as the genes taking part in repairing of DNA are also involved in inducing cancer. It is also important to notice that only 5% to 10% of cancer cases are related to genetic problems (Anand et al., 2008).

A biomarker is termed as an aspect that can be quantified and assessed as an index of normal biological and pathological processes, or pharmacological responses to a therapeutic intervention. The biomarkers of cancer are found in blood and tumour tissues, they contain a broad range of biomolecules such as mRNA, DNA, metabolites, enzymes, transcription factors as well as surface-bound receptors of cell (Sawyers 2008). Numerous methods are used to detect cancer, i.e., ELISA (enzyme-linked immunosorbent assay) (Alberti et al., 2014), SERS (surface enhanced Raman spectroscopy) (Law et al., 2011), PCR (polymerase chain reaction) (Pfitzner et al., 2014), electrophoresis (Lee et al., 2014) as well as electrochemical (Labib et al., 2013) and colorimetric assays (Wang et al., 2012). Nonetheless, these methods are very efficient, but they still lack sensitivity, specificity and accuracy for their clinical diagnostic applications (Wu and Qu, 2015).

In the diagnosis of cancer, protein markers and circulating tumour DNA (ctDNA) are some key biomarkers. Protein markers contain the substances that are generated by cancerous cells or via the cellular response of the cells. They are usually located in blood but they are traced in urine also (Hanash et al., 2008). The applications of protein biomarkers have become very limited because proteins cannot be amplified and they are sensitive to temperature, pH and environmental conditions (Wu and Qu, 2015). Cancer cells are reported to liberate more DNA in plasma than the healthy people. Therefore, ctDNA is a promising biomarker for the detection and assessment of tumours in a non-invasive way (Diehl et al., 2008). But ctDNA method lacks sensitivity, specificity and cannot be available for all the patients (Ma et al., 2015).

In this review article, we will discuss the emerging biomarkers that can be used for cancer detection and treatment, in future. Furthermore, we will address how these biomarkers are opening the doors of research towards precision oncology.

1. Biomarkers

Emerging biomarkers for cancer diagnosis

There are various biomarkers that are used to study the prognosis and diagnosis of cancer. However, we have discussed some most preferably applied biomarkers in cancer detection that are helpful for the application of precision oncology. These biomarkers include circulating tumour cells (CTCs), liquid biopsy, exosomes, non-coding RNAs (ncRNAs) along with metabolic and imaging biomarkers. Figure 1 depicts the biomarkers of cancer discussed in this research paper.

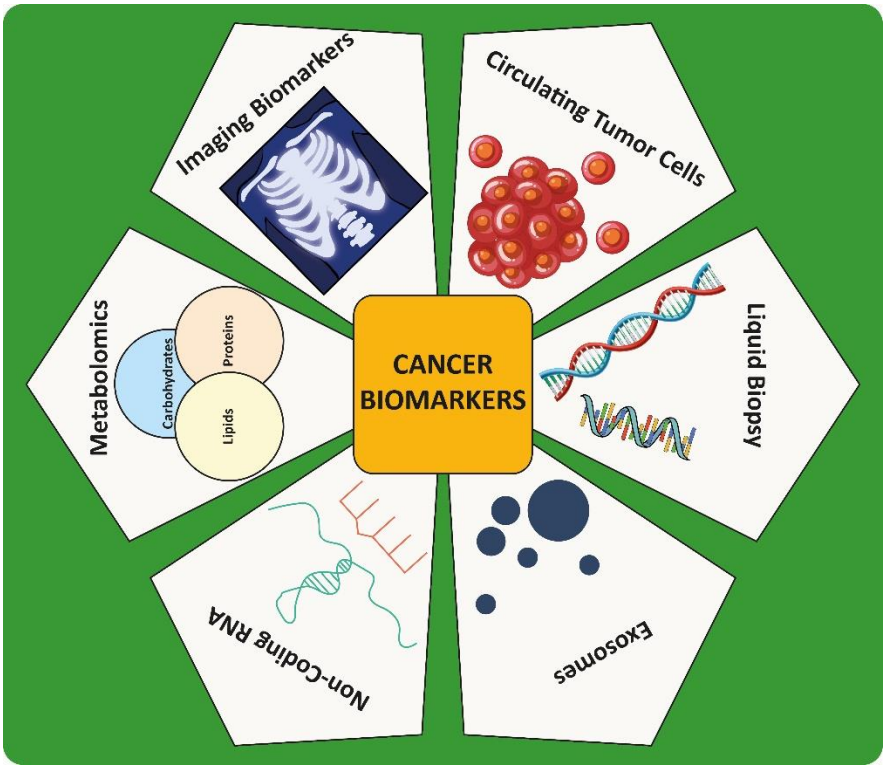

Figure 1: The biomarkers of cancer.

2. Circulating tumour cells

Circulating tumour cells (CTCs) are the cancerous cells that propagate from the primary tumour and move through the bloodstream (Lin et al., 2021). Ashworth (1869) firstly elaborated CTCs after observing “some cells” that are similar to cells found in primary tumours and are present in blood of metastatic cancer patient. CTCs in the bloodstream are the source of metastasis in different organs (Lozar et al., 2019). Figure 2 demonstrates the movement of CTCs in bloodstream. They are not common in blood, but can be spotted and counted by using different methods of separation from blood cells.

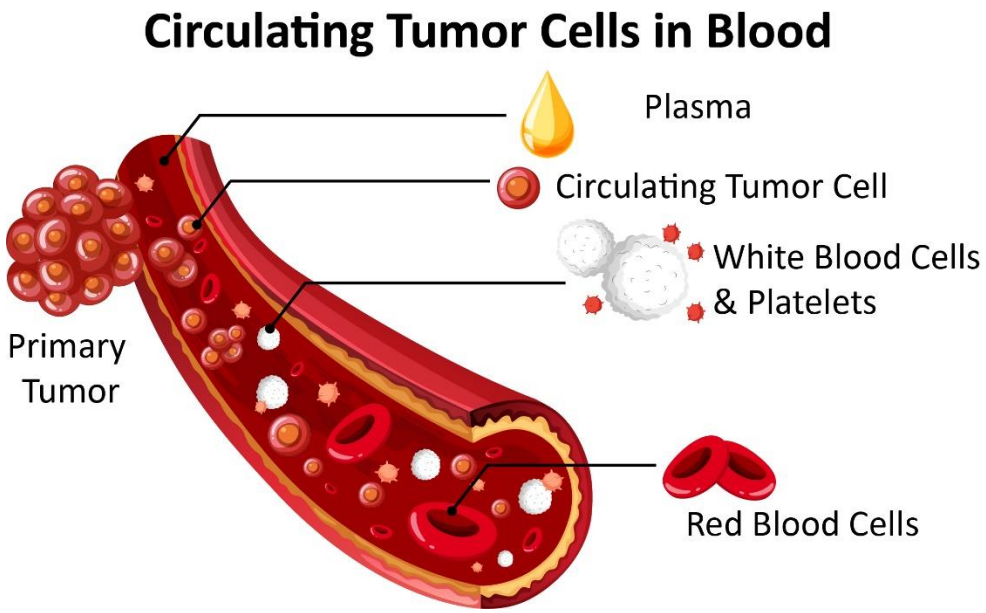

Figure 2: CTCs enter the bloodstream after falling from the primary tumour sites. Through the blood, these tumour cells get access to different areas of the body and causes metastasis. They exist in the form of single cell or accumulate to form a cluster of CTCs, which possess more metastatic potent that single ones. (Designed by Freepik)

2.1. Importance of CTCs in cancer diagnosis

CTCs can serve as a credible biomarker for diagnosing tumour and also help to distinguish between malignant and benign lesions. To achieve the designated energy levels of invasive and metastatic cells, CTCs may cause alterations in metabolic pathways (Pantel and Speicher, 2016). In fact, it has been recently proved that metastatic CTCs have an increased mitochondrial respiration/oxidative phosphorylation which depends upon PGC-1α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha) to encourage mitochondrial biogenesis and oxidative phosphorylation (LeBleu et al., 2014). CTCs are often concealed by billions of other cells, as they are present in peripheral blood in a very low concentration. This has hampered their clinical value as well as the understanding of their process of action. Because of their large morphological differences and the rare presence of CTCs within the blood, the separation & characterization of CTCs can be altogether thought-provoking despite of their clinical relevance (Low and Wan, 2015).

2.2. Recent advancements in CTCs detection technologies

CTCs have been detected by using a wide range of novel detecting techniques by the researchers in recent years. Owing to the low-cost, low limit of detection, specificity, and sensitivity of the target molecule, biosensors have emerged as highly effective tools for CTCs

detection. For the identification and isolation of CTCs, different techniques have been introduced such as antibody-dependent approaches and physical feature-dependent techniques (Sun et al., 2016). In addition to this, a comprehensive understanding of tumour malignancy and oncology therapies including targeted therapy is provided by single-cell next-generation sequencing of CTCs (Kojima et al., 2023). CTCs are distinct from all other known tumour biomarkers and offer a unique source of valuable information about cancer.

2.3. Applications of CTCs in early detection, monitoring, and prognosis

The isolation, quantification and differentiation of CTCs are widely acknowledged for their substantial capability in early cell screening, diagnosing and prognosis. These cells arise from primary tumour & disperse to different places through the bloodstream (Pantel and Speicher, 2016). Over the past decade, many methods of CTCs detection have been formulated, each making use of unique characteristics of CTCs for sensitive selection, enrichment and capture of downstream cellular and molecular description. These profit-oriented approaches can be characterized on the basis of label dependent and label independent methods of CTC identification (Habli et al., 2020). In label dependent method or technology, the CTC identification and separation is immune-based processes wherein the antibodies are utilized to adhere specifically to cell surface antigens (Gorges et al., 2012). Tumour cells can be extracted from the circulatory cells and show several cell surface markers different from those of blood cells. To be specific, CTC does not show CD45 (cluster for differentiation 45), a differential marker for white blood cells, but shows epithelial markers like EpCAM (epithelial cell adhesion molecule) & CK (cytokeratin) to different levels. The antibodies can be modified onto magnetic nanoparticles or constrained on the walls of microfluidic chips for capturing CTCs by the positive selection of CTC or the negative WBCs reduction (Murlidhar et al., 2016; Salar et al., 2024). This technique is also called as affinity-dependent separation and capture of CTC.

While in label-free detection technologies, CTCs show varying degrees of EpCAM & CK expression, with some demonstrating the proper reduction of these proteins, other approaches have been established as well as examined to separate and specify CTCs according to their biophysical properties. CTCs can be differentiated from other cells by using these strategies depending on physical features e.g. electrical properties, deformation, density and size. CTCs identified in cancer patient's peripheral blood may appear as isolated CTCs and circulating tumour microemboli (Zhang et al., 2017). Tumour cells, by losing their intracellular bonding go through epithelial–mesenchymal transformation to infiltrate into the circulation of blood (Kasimir-Bauer et al., 2012). Generally recognized, cancer found at an elevated stage does not remain treatable. Liquid biopsy

includes characterizing CTCs which needs extracting the patient's blood in a small volume and quantifying the numbers of CTCs per unit volume. The applications of CTCs have allowed the diagnosis of different types of cancer i.e., castration-resistant prostate cancer (CRPC), breast cancer and colorectal cancer (Lianidou et al., 2015). Moreover, they can be applied for early diagnosis and prognosis of cancer.

3. Liquid Biopsy Technologies

3.1. Introduction to liquid biopsy

The liquid biopsy is an equipment that permits the execution of personalized therapeutic techniques. Presently, this is one of the most revolutionary methods in tumour study. Its considerable capacity as an apparatus of early detection as well as screening, the capability to judge the status of patient following evaluation and after disease, the efficiency of real-time treatments in different cancer types perfectly demonstrates its advancement. Moreover, liquid biopsies also have the ability to attenuate the drawbacks of tissue biopsy (Fernández-Lázaro et al., 2020). These biopsies involve the sampling and analysis of liquid biological samples, such as blood, for diagnostic purposes, screening, and prognosticating cancer. The tumour circulome represents a subset of circulating units obtained from cancerous tissues. These circulomes are capable of serving as a direct or indirect source of cancer biomarkers in liquid biopsies (De Rubis et al., 2018). The basic elements of this entity consist of circulating tumour nucleic acids (such as ctDNA and ctRNA), circulating tumour proteins, circulating tumour cells (CTCs), tumour-educated platelets and extracellular vesicles. In comparison with typical tissue biopsies, liquid biopsy shows multiple benefits, and technological improvements in sample separation e.g., improvements in the nucleic acid extraction chips to reduce the exploitation of samples, detection platforms (e.g., the advancements of high-resolution flow cytometers) and single-cell western blot platforms are emerging advancements that aid in the application of this technique (De Rubis et al., 2018).

3.2. Diagnostic and prognostic potential of liquid biopsy

Liquid biopsy is a modern diagnostic and prognostic approach in precision tumour study that has taken substantial acknowledgement in recent years for exceeding the current demerits linked with tissue biopsies (De Rubis et al., 2018). For instance, cancer, where there are frequently present in hard-to-reach regions like organs and tissues e.g., in the lungs. It is significantly difficult to take biomarker measurements directly from the tissue because of presence of notable risks, such as, injury, infection and bleeding from invasive biopsies. So, the liquid biopsy has the benefit that

it offers non-invasive and resilient methods to solve this issue (Sholl et al., 2016). From the angle of tumour heterogeneity, executing serial liquid biopsy of entire population of tumour cells is far better option than tissue biopsy sample, this method allows to determine tumour heterogeneity longitudinally as well as facilitates continuous supervision of the tumour genetic profile. Liquid biopsy also involves the separation of entities arising from tumours, such as CTCs, ctDNA, tumour extracellular vesicles etc., from the fluids of body of the cancer patients. Consequently, genomic and proteomic data obtained from these aspects is assessed to determine disease prognosis and treatment (Lone et al., 2022). Sisodiya et al. (2023), figure 3, described the fluids that can be used for liquid biopsy and the components present in them which can be used for early diagnosis and prognosis of cancer. In addition to this, it also covers the applications of liquid biopsy during cancer detection.

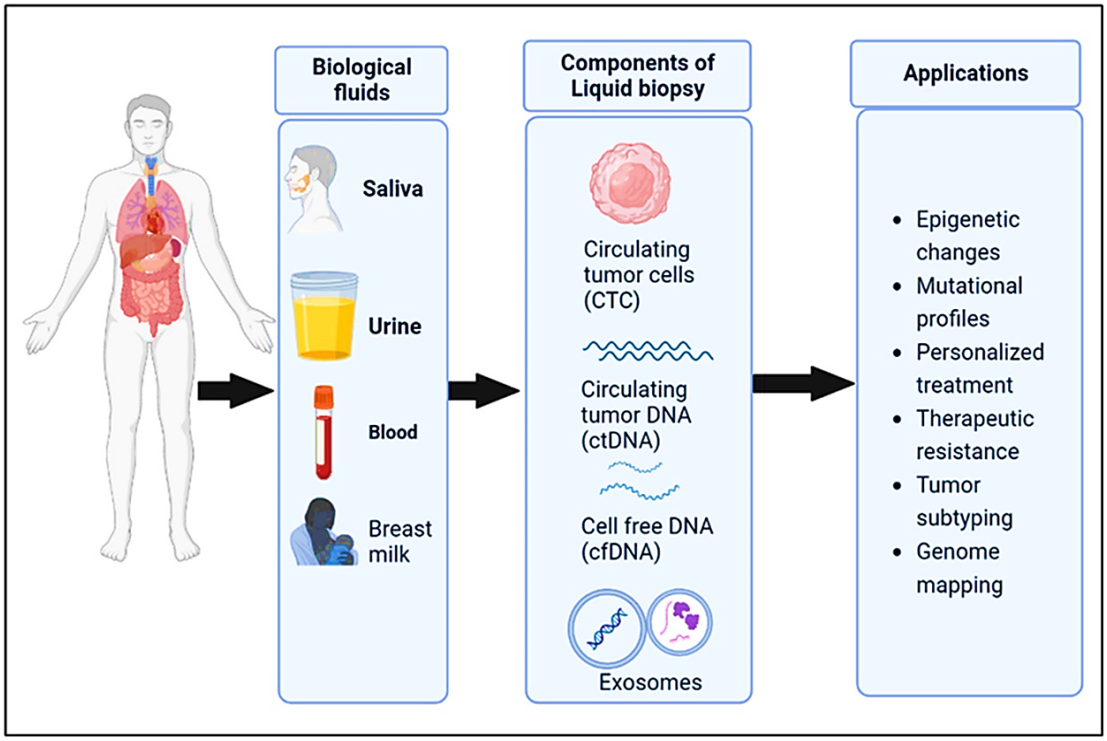

Figure 3: The biological fluids and components of liquid biopsy and their applications that are helpful in cancer detection (Figure copied from Sisodiya et al., (2023), licensed under CC BY-NC-ND, for non-commercial purpose only).

3.3. Recent studies showcasing the effectiveness of liquid biopsy in diverse cancer types

Numerous researches have clearly shown that liquid biopsy is a potential and valuable technique for identifying genetic changes across different types of cancer. These outcomes have determined cancer-specific markers like KRAS (Kirsten Rat Sarcoma viral oncogene homolog), CEA (Carcinoembryonic Antigen), ERBB2 (avian erythroblastic leukemia viral oncogene

homolog 2), EML4 (Echinoderm Microtubule-associated Protein-Like 4)-ALK (Anaplastic Lymphoma Kinase), EGFR (Epidermal Growth Factor Receptor) and Septin-9 in the fluids of body of patients. Furthermore, they have demonstrated the viability of reconstructing tumour genomes by using DNA of plasma (Bettegowda et al., 2014). As the liquid biopsy can be done continuously during the check-up, this can be utilized for checking therapeutic response and prognosis (Brooks 2012).

Mutations in genes like KRAS, v-Raf murine sarcoma viral oncogene homolog B (BRAF), Tumour Protein 53 (TP53), Adenomatous Polyposis Coli (APC), Carcinoembryonic Antigen (CEA), and Septin-9 are common occurrences in colorectal cancer (Sun et al., 2021). The analysis of mutations in these genes by using liquid biopsy act as a cancer screening tool in populations in danger of cancer and is being studied now-a-days. Conversely, to breast and prostate cancer, colon cancer patients show minimum degrees of CTCs in peripheral blood circulation, putting forward challenges for their detection (Diehl et al., 2005). Liquid biopsy has been studied as an approach for examining the staging and prognosis of colon cancer. TNM (Tumour, Node, Metastasis) staging demonstrates a clear correlation with tumour features present in the patient's blood samples (Lefebure et al., 2010).

Current advancements in different digital genomic techniques and next generation sequencing (NGS) approaches, promote the clinical usage of finding CTCs, ctDNA, circulating cell-free DNA (cfDNA), circulating cell-free RNA (cfRNA) which consists of primarily small RNAs but also mRNAs, tumour-educated platelets (TEPs), circulating extracellular vesicles (EVs) such as exosomes, proteins, and metabolites usually found in the plasma of cancer patients and particularly in breast cancer to be used as a liquid biopsy (Low et al., 2018). These detections based on blood of genetic biomarkers (e.g., ctDNA, cfDNA etc) effectively play their part in the pathogenesis, screening, aetiology, detection, survival and prognosis of breast cancer. It also includes the assessment of response to treatment and giving primary data for clinical determination by a cost effective, minimally invasive and clinically applicable technique (Openshaw et al., 2016).

3.4. Challenges and future directions in liquid biopsy research

Because of some drawbacks of liquid biopsy, its clinical use is obstructed e.g., lack of diverse standardization, less specificity and sensitivity, and elevated prices for tests and separation protocols. With recent technology, we gain an understanding of tumour activity and gene expression at a fundamental level. The technology should be upgraded by use of multi-organ cancer identification and in-depth tumour examination. The integrated usage of liquid biopsy in

technology and research can enable its ideal applications in clinical setups specially cancer (Lone et al., 2022). Efforts should be made to enhance the cost-efficiency of this technique to facilitate its broader adoption in clinical practice.

4. Exosomes as Biomarkers

4.1. Overview of Exosomes

Exosomes, ranging from approximately 30 to 200 nm in diameter, are diminutive organelles characterized by a single membrane, and structure similar to that of cell (Pegtel and Gould, 2019). Exosomes embrace various cellular components, comprising lipids, DNA, RNA, metabolites, cell-surface, cytosolic proteins (Kalluri and LeBleu, 2020) as well as glycoconjugates (Pegtel and Gould, 2019). Exosomes are formed as a consequence of budding at endosome and plasma membranes. They contain a diverse range of membrane-associated, high-order oligomeric protein complexes, that show significant heterogeneity in molecules. Exosome biogenesis serves as a mechanism for maintaining protein quality control, and upon release, exosomes conduct several procedures such as, extracellular matrix remodeling as well as the transmission of signals and molecules to neighboring cells. This channel of intercellular vesicle transport plays pivotal part in various perspectives of human physiology and pathology, encompassing developmental processes, immune responses, tissue equilibrium, cancer progression, and neurodegenerative conditions (Pegtel and Gould, 2019).

4.2. Role of exosomes in intracellular communication:

Exosome absorption and secretion routes can overlap and result in the cumulative production of a diverse population of exosomes over a period of time for any specific cell. This includes both endogenously produced as well as recycled exosomes (Kalluri and LeBleu, 2020). There are various mechanisms and pathways involved in exosome uptake (Mathieu et al., 2019) coupled with the potential selectivity of exosomes for specific types of cells, which contribute to the intricate role of exosomes in cell-to-cell communications (Kalluri and LeBleu, 2020). For instance, in human pancreatic oncogenic cells cancerous signals instigated as a result of mutation in KRAS (Kirsten rat sarcoma viral oncogene homolog) expression facilitate uptake of exosomes via macropinocytosis (Kamerkar et al., 2017).

The melanoma cells in human acquire exosomal cargo via their integration with the plasma membrane of the cell. Exosomes transport peripherally associated surface proteins, out of which many take part in the signaling (Pegtel and Gould, 2019). Furthermore, the surface of exosomes is saturated with the proteins of extracellular matrix, i.e., tenascin C, fibronectin and extracellular

matrix protein 1 (ECM-1). These proteins contribute significantly to adhesion as well as signaling (Santasusagna et al., 2018). Moreover, protein abundant inner cortex of exosomes facilitates the connections between membrane proteins of exosomes through their cytoplasmic tails. In addition to this, they are also involved in the linking of other proteins, partner proteins as well as lipids (Pegtel and Gould, 2019).

4.3. Significance of exosomal biomarkers in cancer diagnostics

Exosomes are associated with the innate immune response in cancer (Kalluri and LeBleu, 2020). It has been reported that normal human blood typically contains around 2 quadrillion exosomes, whereas this number escalates to about 4 quadrillion in the blood of cancer patients. Furthermore, damaged organs and the aberrant cells within them are known to produce even higher quantities of exosomes (Melo et al., 2015). In cancer, exosomes have primarily described as promoters of tumour growth (Melo et al., 2015); nevertheless, it is possible that exosomes can have antitumour capabilities and serve as inhibitors of the progression of the disease (Kalluri 2016). Exosomes present in pancreatic tumour cells and patients are reported to mitigate complement-regulated lysis and reduce cytotoxicity against oncogenic cells (Capello et al., 2019).

The research on exosomes in cancer has advanced at a faster rate than research into their role in other medical conditions (Kalluri 2016). Exosomes have been linked to various cancer specific traits, i.e., tumour growth, neoplasia, and metastasis, therapeutic resistance and paraneoplastic disorders. It is important to understand that the function of exosomes in the malignancy progression is specific as well as dynamic in context of the type of cancer, genetics and stage. Exosomes originating from pancreatic malignant cells have been proved to begin cell transformation via prompting mutations (Stefanius et al., 2019). The transport of exosomal miRNA (microRNA) cargo from the prostate and breast cancer cells can also cause neoplasia. Furthermore, miR125b (microRNA-125b), miR-130, miR-155, along with HRas (Harvey Rat sarcoma virus) and Kras (Kirsten rat sarcoma viral oncogene homolog) mRNAs in exosomes derived from prostate tumour cells, actively engage in neoplastic reprogramming as well as production of tumours in adipose stem cells (Abd Elmageed et al., 2014). Exosomes add versatility to cancer cells and aid in the malignant progression i.e., transformation from epithelial to mesenchymal transition in breast cancer cells (Le rt al. 2014). Figure 4 illustrates the movement of exosomal cargo from tumour cells to normal cells and its adverse effects.

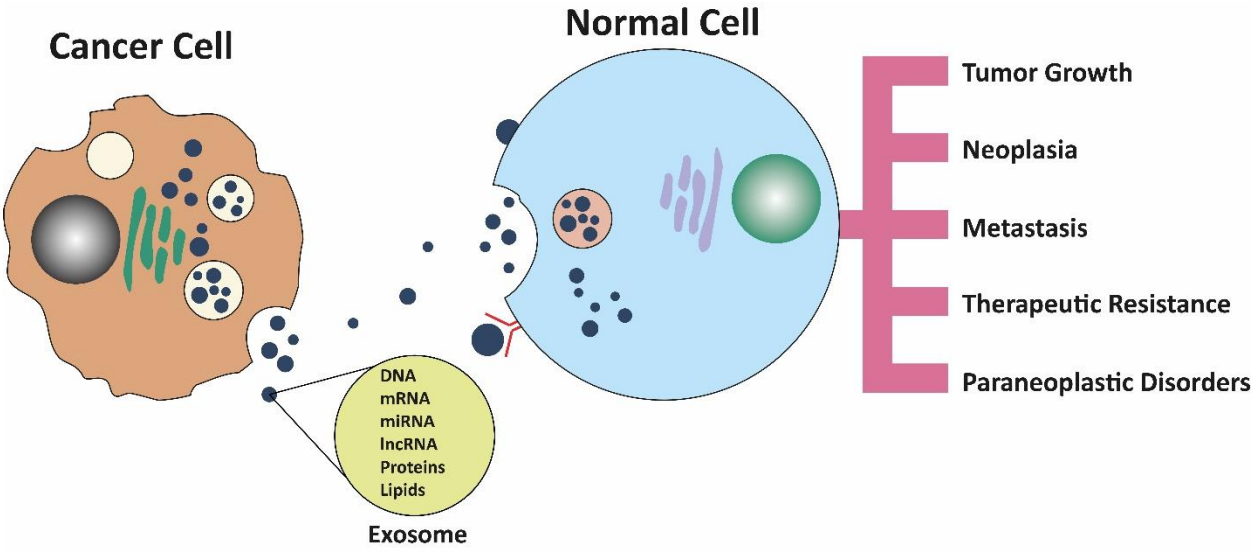

Figure 4: Initially, an invagination occurs in the cancer cell which form an endosome. Following this, the selection of endosomes occurs and exosomal cargo i.e., DNA, mRNA, miRNA, lncRNA, proteins and lipids are encapsulated in multivesicular bodies. Then, these multivesicular bodies fuse with plasma membrane and the exosomes are released into extracellular space. These exosomes get access to the recipient normal cells via endocytosis, receptor interaction as well as cell membrane fusion (Tan et al., 2021). In this process, the tumour cells proliferate the normal cells also and lead to deleterious effects.

Exosomes are reported in various fluids of body, including blood, urine, breast milk, semen, tears, saliva, ascites, amniotic fluid, bile, and cerebrospinal fluid. Cancer exosomes hold potential as a liquid biopsy tool for diagnosing malignancies, including prostate, pancreatic, breast, and ovarian cancers; glioblastoma; and melanoma (Melo et al., 2015). Elevated concentrations of exosomes have been reported in the systemic circulation of patients diagnosed with ovarian, breast, and pancreatic cancer (Melo et al., 2015). Identifying specific markers linked to cancer exosomes can improve their enrichment and may be serve as a useful tool for diagnosis when harvested from a heterogeneous population of exosomes in biological fluids. Numerous in vitro and preclinical researches have contributed to our comprehension of exosome research and its potential applications in cancer detection and monitoring. While lipids and metabolites present in cancer exosomes may also provide unique insights into cancer detection and biology, more precise knowledge is evolving regarding the utility of proteins and nucleic acids in exosomes (Kalluri, 2016).

The knowledge of the contribution of exosomes in diseases still continues to evolve, with a growing number of researches focusing on their potential in the detection and cure of multiple

disorders. The diversified cargo of exosomes provides a wide array of diagnostic information, offering a valuable tool for the detection as well as monitoring of disease. In fundamental and clinical practice, the unique characteristic of exosomes in providing functional cargos to affected cells also support their part as therapeutic interventions in cancer study (Kalluri and LeBleu, 2020). Exosomes can therefore provide important information about cancer and play its part in cancer control and precision oncology.

5. Non-coding RNAs

5.1. Overview of non-coding RNAs and their types

Non-coding RNAs (ncRNAs) have been acknowledged as a potential biomedical tool for nearly six decades, dating back to the 1950s when transfer RNA and ribosomal RNA were identified (Palazzo and Lee, 2015). During the late 1970s and early 1980s, researchers found different functional non-coding RNAs (ncRNAs), such as Ribonuclease P (RNAse P), small nuclear RNA (snRNAs), and 7SL (signal recognition particle RNA). The concept of non-coding RNAs (ncRNAs) is generally utilized to elaborate RNA molecules that do not encode proteins. However, this does not mean that these RNAs lack information or are non-functional. The general assumption held that most genetic information is executed by proteins. According to recent research, the major portion of the mammalian and other complex organism genomes is transcribed into ncRNAs, many of which undergo substitute splicing and processing into smaller products (Mattick and Makunin, 2006). The intricate regulatory networks, tissue-specific expression patterns and evolving roles collectively underscore the value of ncRNAs as more than only byproducts of transcriptional processes or aberrant splicing; instead, they are significant regulatory molecules (Palazzo and Lee, 2015). Current technological processes have supported the achievement of various goals in ncRNAs identification across different study disciplines. These techniques consist of detecting ncRNAs expression on a transcriptome-wide scale, identifying new varieties of ncRNAs, exposing biomarkers related to diagnosis of cancer and assessing potential functional RNAs within particular subcellular compartments. Moreover, as research on cancer-related ncRNAs expanded, translational usage involving the identification of particular ncRNAs for clinical diagnosis has appeared, including the development of diagnostic kits (Sun and Chen,2020).

ncRNAs can either be Long non-coding RNAs (LncRNAs) or MicroRNAs (miRNAs), figure 5(a). LncRNAs represent the most predominant and functionally diverse category of ncRNAs. LncRNAs are a group of RNA transcripts, characterized by their length, typically exceeding 200 nucleotides. LncRNAs show secondary and 3-dimentional structures, which allow

them to function as both RNA and protein (Chi et al., 2019). LncRNAs are ubiquitously present throughout various taxonomic groups including animals, plants, yeast, viruses and even prokaryotic organisms (Taneja and Upadhyay, 2021). Unlike other well-researched RNA molecules, i.e., mRNA, miRNAs and small nuclear RNAs, LncRNAs show limited conservation across various species. However, LncRNAs play vital role in various important biological processes including splicing, transcription, translation, cellular structure integrity, cell cycle, imprinting, and programmed cell death, as well as heat shock response (Heydarnezhad et al., 2022). Studies have reported that LncRNAs may modulate the progression of cancer and development of many other human diseases (Tano and Akimitsu, 2012).

LncRNAs take part in almost every characteristic feature of cancer cells, including their inherent capacity to proliferate and survive, increased metabolic action, and their interactions within the tumour microenvironment (Statello et al., 2021). The first signs of LncRNAs involvement in cancer evolved from their modulation of transcription by key oncogenic or tumour-suppressive transcription factors including, p53 (cellular tumour antigen), oestrogen receptor, signaling cascades such as the Notch pathway or MYC Proto-Oncogene (Huarte 2015). These LncRNAs impact the functionality of the tumour-suppressive responses and P53 triggers some LncRNAs as a result of DNA damage (Statello et al., 2021), figure 5(b). However, there is broad consensus that LncRNAs play a pivotal role in cellular differentiation, growth and the etiology of several ailments, including cancer. LncRNAs have a crucial role in the development of several oncogenic tumours by enhancing cellular migration, proliferation, as well as disruptions at the stage of transcription, translation, and post-translation. Various LncRNAs play key roles in the aetiology of diverse malignancies, suggesting that they could be used as a novel therapeutic target (Hu et al., 2018).

MicroRNAs (miRNAs) are non-coding RNA molecules that usually have a length of 8-24 nucleotides and regulate target mRNAs. In 1993, initial microRNAs (miRNAs), lineage abnormal-4 (lin-4) and lethal-7 (let-7) were discovered. The function of microRNAs (miRNAs) in regulating various biological mechanisms such as proliferation, apoptosis, differentiation, and embryonic development is well established in multicellular eukaryotes. It has been reported that more than half of microRNA (miRNA) genes are found in fragile genomic sites and areas related to cancer, indicating a pivotal role of miRNAs in the development of human cancers. Making use of this diversity of microRNAs (miRNAs) is important for progress of new therapeutic methods in human medicine. miRNA expression profiling has been found to be related to disease pathogenesis and

prognosis, offering promising opportunities for future uses in managing human tumours (Manikandan et al., 2008).

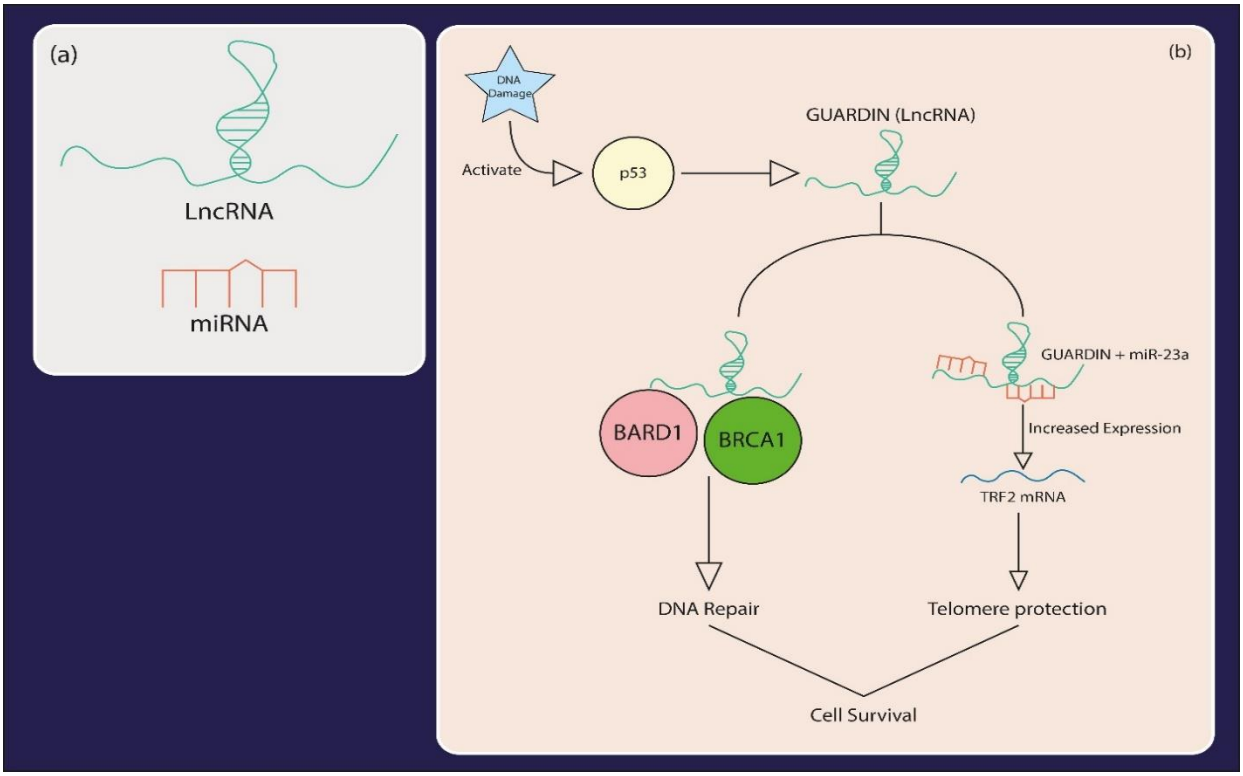

Figure 5: (a) Diagrammatic illustration of LncRNA and miRNA. (b) DNA damage provokes p53 which in response produce GUARDIN, a LncRNA. GUARDIN can take two paths, either it can promote the interaction of BARD1 and BRCA1 to form a protein complex that is important for DNA repair. Or it can bind with miR-23a (a microRNA) and promote the expression of TRF2 mRNA, which protects the telomeres. Both of these pathways are indispensable and play a major role in the DNA repair and cell survival.

5.2. Regulatory roles of ncRNA in cancer pathways

Because of their different genetic expression profiles in malignancies and exceptional stability in urine, plasma or saliva, ncRNAs (particularly LncRNAs, miRNAs, and circular RNAs), are increasingly recognized as promising non-invasive bioassays for cancer diagnosis. The advancements in research have resulted in the recognition of appropriate ncRNAs markers for malignant diagnosis. In addition to this, multiple ncRNAs diagnostic kits for the diagnosis of cancer have been applied in clinical practice. Whereas, some of promising ncRNAs tools are undergoing pre-clinical evaluations (Sun and Chen, 2020). Furthermore, conflicting findings in studies assessing the diagnostic potential of ncRNAs have emerged (Wang et al., 2019). To

reconcile these disparities, more precise assessments of RNA expression patterns in larger clinical cohorts are mandatory (Sun and Chen, 2020).

The Mitogen-activated protein kinase (MAPK) signaling pathway is found to be associated with liver cancer. ncRNAs act as a cornerstone in the development of liver cancer. ncRNAs take part in multiple MAPK signaling pathways during hepatocellular cancer and modulate the expressions of proteins linked to the extracellular signal-regulated kinase (ERK) and Jun N-terminal kinase (JNK) signaling pathways. Therefore, the association of ncRNAs with MAPK signaling pathways demonstrates that it can be applied as a biomarker for cancer therapy including liver cancer treatment (Wang et al., 2022).

The nuclear factor-kappa-B (NF-κB) signaling system is another potential therapeutic target of tumour therapy. Various stimuli can activate the NF-κB signaling pathway under both healthy as well as diseased conditions. NF-κB has been found linked with ncRNAs in various types of cancer. Various studies have suggested that miRNAs can hinder or instigate NF-κB signaling in tumour models. miRNA/ NF-κB axis has been found to be linked with cancer growth, metastasis as well as response to radiotherapy and chemotherapy. NF-κB is important for cancer cell growth and instigates angiogenesis, proliferation and migration (Mirzaei et al., 2021). It is also found to modulate telomerase which is crucial enzyme involved in cancer growth (Ozturk et al., 2017). However, anti-tumour agents that have the ability to suppress NF-κB signaling are helpful in cancer treatments. Moreover, ncRNAs can be used as biomarkers due to their association with NF-κB signaling pathways.

5.3. Clinical Applications of ncRNAs

It is widely accepted that LncRNAs act as bioindicators of cellular conditions and depict the actively occurring processes in the cell. Furthermore, they hold potential for identifying cellular abnormalities as cancer, providing prognostic value, and informing reliable therapeutic techniques for the cure of cancer patients (Schmitt and Chang, 2016). Recent investigations have demonstrated the significance of LncRNAs in determining initial stage of prostate cancer and also found to be linked with metastases in lymph nodes. Moreover, emerging evidences suggest that LncRNAs may predict the responsivity to particular kind of tumour therapy. The quick advancement of bioinformatics and sequencing studies have revealed many novel kinds of ncRNAs, out of which some play critical part in cancer prognosis (Wang et al., 2022). Consequently, medications that target these ncRNAs have the potential to treat cancer.

5.4. Challenges in utilizing ncRNA as biomarker

The clinical development of all RNA-based therapies has been hindered by challenges related to specificity, delivery as well as toxicity. Specificity issues consist of undesired on-target effects generating from uptake in cells other than the target cells, along with off-target effects resulting from sequence analogies or excessive endogenous degrees at importantly increased concentrations.

The primary factors that lead to delivery related concerns include, the instability of "naked," chemically unmodified RNA structures; their ineffective intracellular delivery, demanding the use of endosomal escape procedures; and the absence of proper delivery cargos for targeting specific organs and cell types of interest. The pathogen-associated molecular pattern (PAMP) receptors including Toll-like receptors, produce an immune response against these RNA structures and causes tolerability concerns (Winkle et al., 2021).

Additionally, as many RNA therapies rely upon the endogenous RNA machinery, excessive dosing might result in saturation of the system and which will stop endogenous miRNAs from functioning (Winkle et al., 2021). The initial report on this process analyzed that strong shRNA (Short hairpin RNA) overexpression in hepatocytes led to reduction of miRNAs resulting in hepatic damage and death in mice. One of the most challenging issues in the field lies in efficient transport of RNA therapies not only to the targeted organ and cellular type but also across the cell membrane to attain their intracellular functions. Resultantly, the primary reason for terminating clinical trials linked to RNA therapies is often due to their lack of efficacy. The efficient transportation of oligonucleotides indicates difficulties due to their instability, negative charge, and hydrophilic nature, which impede their diffusion across cell membranes (Baumann and Winkler, 2014)

6. Metabolomic Biomarkers

6.1. Introduction to metabolomics in cancer research

Metabolites are the byproducts and substrates of metabolism that power vital cellular processes like production as well as storage of energy, apoptosis and signal transmission. Metabolites are not only produced straightforwardly by host organisms but also synthesized by xenobiotics, microorganisms as well as exogenous and dietary sources (Johnson et al., 2012). For decades, few metabolites have been applied for diagnosis of disease and have influenced clinical treatments i.e., determination of phenylalanine in infants in order to identify phenylketonuria and the creation of strips to test blood glucose level in 1950s to screen for diabetes). Metabolomics is the extensive study of metabolic products in an organism at individual, organ, tissue and cellular

level. It can also be defined as a systemized investigation of exclusive chemical markers that are left behind by some distinct cellular processes (Krishna and Krishna, 2021). In metabolomics, the metabolites that are extensively researched include, small lipids, amino acids, co-factors, monosaccharides, nucleotides, intermediates of energy cycles and xenobiotics (Clish 2015). The essential factors that play important role in maintaining metabolism, which are crucial for life, are redox balance, energy and biomass. Metabolites provide a non-invasive method for the regulation of cancer patients as they can be determined by taking samples from body fluids including urine, plasma and blood, therefore, delivering some unique biomarkers for tumour diagnosis and progression. Furthermore, it can predict the effect of anticancer cure at an individual level (Belhaj and Lawler, 2021).

Reportedly, the alterations in the metabolism are responsible for cancer development (Gallagher and LeRoith, 2015). Nowadays, metabolomic indices are applied in clinical care for the diagnosis of cancer i.e., prostate cancer. For instance, ^{18}F-fluorodeoxyglucose and ^{18}F-fluorocholine are radiotracers used to detect prostate cancer by identifying increased choline and glucose metabolism in cancerous tissues using positron-emission tomography (PET) (Vali et al., 2015). The research methodology includes determination of metabolites by applying high-resolution and high-throughput detection technology, obtaining enormous datasets, gaining various metabolites by identifying metabolic pathways, data analysis as well as elucidating their biological significance. Gas chromatography-mass spectrometry (GC-MS), Liquid chromatography-Mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) are some metabolomics techniques that are normally applied (Han et al., 2021).

6.2. Identification of metabolic signatures associated with cancer

Metabolomics is an organized biological approach for the determination of alteration in the endogenous small molecular metabolites. It is widely applied in disease diagnosis, disease biomarkers discovery and study of disease pathogenesis (McCartney et al., 2018). Tumour cells are capable of adapting to various metabolic pathways and also exhibit heterogeneous expression of genes encoding metabolic enzymes. The physiological needs of origin of cancerous cell and the host tissue are assessed to ascertain the metabolic adaptations. Various metabolism mediators that are involved in cancer cell proliferation include unconstrained metabolism of amino acids, fatty acids, glucose, nucleotides, microRNAs, tumour suppressor genes as well as numerous modulatory genes and enzymes (Kumar et al., 2022).

In 1920s, Otto Warburg recognized that cancer tissues continue to uptake glucose and produce lactate at rapid pace even when enough oxygen is available i.e., aerobic glycolysis (Warburg 1925). Perhaps, lactate is the only metabolite that is associated with malignant tumour progression. Lactate is absorbed by normoxic cancer cells and used as a fuel for oxidative phosphorylation, therefore, limiting glucose acquisition which is swiftly utilized by hypoxic (low level of oxygen) cells (Romero-Garcia et al., 2016). The main feature of metabolic reprogramming during cancer is switching towards aerobic glycolysis. Immediately after hypoxia, it disrupts the function of suppressor genes and improves the performance of oncogenes. So that, tumour can meet its synthetic and energetic demands required for its growth and progression. Advance molecular studies have proposed that, Steep incline in the rates of aerobic glycolysis which is required for uncontrolled growth is a consequence of aggregation of certain signaling pathways. These pathways are either disrupted by tumour microenvironment or changed by gene mutations or alterations in gene expressions (Romero-Garcia et al., 2016).

In addition to this, altered amino acid metabolism also plays a significant part in the prognosis of cancer. The upsurge in the metabolism of glutamine is the key metabolic change during carcinogenesis. Glutamine is considered the second most significant nutrient after glucose for cancer prognosis in several types of cancer (Kumar et al., 2022). Glutamate is the source of carbon and nitrogen in various activities of cancer cells such as, macromolecule production, signal transduction and energy synthesis. Myelocytomatosis oncogene (c-Myc) is an oncogene transcription factor that elevates the production of glutaminase and metabolism of glutamine in tumour cells (Feun et al., 2015). Glutamine is the major energy source that supports quickly growing cancer cells in fulfilling the elevated demand for reducing agents, ATP and biosynthetic precursors. Moreover, rat sarcoma (Ras), Protein kinase B (Akt) and Adenosine monophosphate-activated protein kinase (AMPK) are some signalling agents that instigate glycolytic enzymes and encourage the production of lactate (Warburg effect). This leads the cancer cells to utilize glutamine metabolism so that they can satisfy their energy demands (Kumar et al., 2022).

In addition to the disrupted glucose and amino acids metabolism, tumour cells also affect fatty acid metabolism. Cancer cells lead to production of fats at elevated levels to support their growth. Tumour cells utilize lipids to produce signalling molecules, ATP and membranes (Corn et al., 2020). During the hypoxic conditions, lipid production is an important pathway for the transfer of electrons in the excessively proliferating cells. ATP citrate lyase is a crucial enzyme that connects glucose metabolism with lipid production. It catalyses the transformation of citrate to cytosolic acetyl-CoA. ATP citrate lyase protein aggregation has been found in prostate, breast,

colon and lung cancer (Nava and Madrigal, 2022). Fatty acid synthase (FASN) is another enzyme that leads to poor prognosis in people suffering from cancer as well as its accretion is linked with higher risk of cancer (Corn et al., 2020). It has been reported that FASN shares homologous enzymology and sequence with oncogenic antigen-519, a major molecular player associated with poor prognosis in breast cancer patients. FASN protein aggregation is found in ovarian cancer, prostate cancer, breast cancer, colorectal cancer, non-small cell lung carcinoma and endometrial carcinoma (Nava and Madrigal, 2022). Figure 6 illustrates the metabolic changes brought about by cancer cells in glucose, amino acids and fatty acids metabolism.

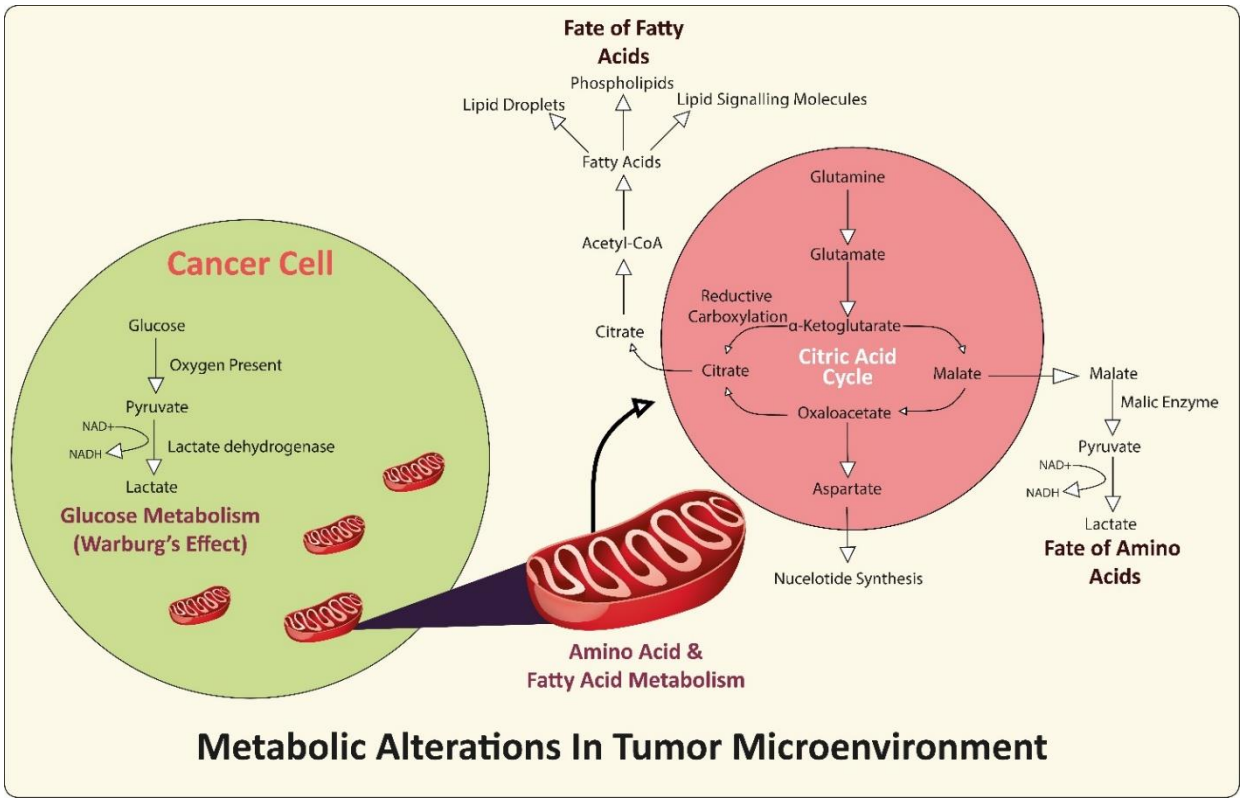

Figure 6: Diverse metabolic changes are made by cancer cells in the tumour microenvironment. In cytosol, the glucose in converted into lactate even in the presence of abundant oxygen (Warburg's effect). Moreover, the metabolism of amino acids and fatty acids is also changed in the mitochondria during the citric acid cycle (TCA). The intake of glutamine is increased which is converted into glutamate and then into α-ketoglutarate. The α-ketoglutarate is then transformed into malate which in the presence of malic enzyme forms pyruvate and subsequently making lactate (energy source for tumour cells). Whereas, α-ketoglutarate is also converted into citrate via reductive carboxylation which is then converted into acetyl-CoA. The acetyl-CoA ultimately forms fatty acids which is then transformed into phospholipids (for membrane formation), lipid signalling molecules and lipid droplets (for energy). Furthermore,

oxaloacetate is converted to aspartate, which is involved in the synthesis of nucleotides. This process provides nucleotides for the proliferation of cancer cells. Moreover, many NAPH molecules are formed. (Mitochondria in figure is designed by Freepik).

Most cancer cells exhibit notably elevated anabolic pathways, including fatty acid synthesis, glutaminolysis, the pentose phosphate pathway (PPP), and glycolysis. This metabolic profile stands in stark contrast to the metabolic processes of normal cells, which rely exclusively on catabolism (oxidative phosphorylation) to generate the energy necessary for cellular homeostasis (Kumar et al., 2022). Cancer cells not only produce ribonucleotides and phospholipids but also synthesize PPP which is a major originator of NADPH. NADP is an important antioxidant that plays role in adaptation of cellular redox reactions. Furthermore, the reduction in mitochondrial oxidative phosphorylation lowers the synthesis of reactive oxygen species which are lethal for tumour cells. The above given details show that how cancer affect the whole metabolism of the body and encourages the malignant tumour progression. The metabolic pathway exhibited by Warburg effect in tumour cells could be helping premature cancer detection in various types of cancer. Hence, techniques for monitoring metabolism are valuable for diagnosing and prognosing cancer (Nava and Madrigal, 2022). The knowledge of cancer has progressed substantially in the past few decades and is being applied to develop unique tools for cancer diagnosis and targeted therapies.

6.3. Applications of metabolomic biomarkers in early detection and treatment response

The applications of metabolomics include cancer research and clinical oncology. Metabolic profiling is a commonly applied method to recognize possible bioassays. It is the examination of predetermined metabolites to identify specific biochemical profiles. Metabolomics serves as a valuable data source in predicting cancer development, assessing response to therapy, and evaluating the risk of recurrence. Metabolomics and its applications have significant practical implementations in the field of precision medicine. In metabolomics, metabolic phenotyping and profiling techniques are helpful in medicating the patients by recognizing the fundamental molecular disruptions that lead to the specific diseased condition. In addition to this, metabolomics is also helpful in forecasting the drug response (pharmaco-metabolomics) (Cheung et al., 2019). Pharmacometabolomics technique is used during organ transplantation to determine the dose of mass spectrometry quantitation of immunosuppressant drugs as well as their metabolites.

High resolution magic angle spinning nuclear magnetic spectroscopy (HR-MAS-NMR) is a method applied to identify metabolic alteration in the cancer tissue samples. It is a non-invasive

technique and possesses a high-resolution spectrum. It is applied in open profiling as it detects the distinct metabolites which can be interpretative bioassays of cancer. It is used to find metabolic differences in tumour and normal cells. For instance, it is applied to find difference between neural ectodermal and glial tumours in kids (Wilson et al., 2009). The HR-MAS-NMR technique is employed to obtain metabolic profiles of both malignant and non-malignant tissues during prostate and colorectal cancer. The alterations in the levels of metabolites show the function of metabolic enzymes in tumour cells. These are helpful in determining the changes at protein, RNA and DNA levels that contribute to functional disruptions in cellular activity. For instance, oncometabolite D-2- hydroxyglutarate which was detected to be significantly raised in cells exhibiting cancer-linked isocitrate dehydrogenase (IDH) mutations. This metabolite was also found to be notably increased in plasma, cells and tissues from cancer having somatic mutations in IDH (Dand et al., 2009). Therefore, metabolic biomarkers play a significant role in early disease diagnosis and precision medicine.

6.4. Integration of metabolomics into precision oncology strategies

It should be noted that, metabolomics is significantly different from other oncology strategies, as no single metabolomics methods can holistically provide the information about cancer and its underlying causes. Therefore, metabolomics techniques should be applied in integration with other omics techniques to identify functionally as well as diagnostically appropriate changes in cancer cells (Schmidt et al., 2021). The combination of metabolomics and other oncology techniques is of great importance as it can explain the pathways of molecular mechanisms involved in tumorigenesis. The incorporation of all these omics techniques has significant importance in the suitable application of personalized medicine since a metabolite links the subsequent target to a specifically marked gene.

Various studies have involved the integration of metabolomics with genomics. For example, the phosphorylated oncogenes MYC and Akt serine/threonine kinase 1 (AKT1) overexpression was found to be correlated with specific metabolic pathways (Moez et al., 2018). Gene-metabolite profiles are helpful in determining gene function by using metabolomic profiles and coordinated gene expressions. Therefore, metabolites can detect a specific gene target that leads to gene annotations. In addition to this, metabolomics has provided significant information about the molecular profile of clear cell renal cell carcinoma). The examination exhibited the linkage between and within metabolomics, phosphoproteomics and transcriptomics as well as the drug targets in clear cell renal cell carcinoma (Dugourd et al., 2021). These studies show the significance of metabolomics integration with other multiomics and personalized medicine

techniques. Briefly, we can say that personalized medicine can only be successful if all the multiomics techniques are applied in association with one another.

7. Imaging Biomarkers

7.1. Introduction of imaging biomarkers and advanced imaging technologies

Imaging biomarker is an attribute derived from the images of a person that can be quantitatively assessed and serves as a marker of normal biological and physiological process, a disease or an outcome of therapeutic measures. There are two key benefits of imaging biomarkers. Initially, they measure variables quantitatively that represent and measure various parameters, attained from medical images which are related to any certain disease. Subsequently, these images permit us to examine the bioassays spatial pattern in the sample that can be examined via its graphical display. These images are produced to deliver a visual presentation of the values of each parameter or biomarker (Bonmatí et al., 2012). Since the inception of radiology, its standard target was to present high-definition images for precise diagnosis. Imaging markers play substantial role in predicting the medical outcomes, i.e., illustrating anomalies, estimating prognosis and grading diseases on the basis of severity. Therefore, radiologists developed a new method of imaging biomarkers to simplify the decision making for personalized medicine (Kang et al., 2015).

Nowadays, medical imaging is crucial application in clinical therapies and diagnosis. It comprises non-ionizing radiation (Magnetic resonance imaging [MRI]) as well as ionizing radiation (Computed tomography [CT], Positron emission tomography [PET] and X-rays) techniques (Chiu and Yen, 2023). PET is an exceptional non-invasive approach of imaging that possesses extensive research and clinical utilities in the determination of abnormal physiology of various brain anomalies, i.e., infection, seizures, neurodegenerative diseases, epilepsy and brain cancer, as well as for studying normal physiology of brain. PET is an ultra-sensitive imaging method that permits the 3-dimensional passage of positron-emitting radiopharmaceuticals given in microscale amount without inducing any pharmacological or physiological impacts (Lameka et al., 2016). In the beginning, PET was regarded as a dynamic tool for the estimation of physiological processes. However, in 1970s, the perspective of PET was significantly widened, following the development of ^{18}F-fluorodeoxyglucose to study metabolism of glucose in brain (Sokoloff et al., 1977). The process of PET imaging starts with the administration of positron-emitting radioisotopes to labelled radiopharmaceuticals. Following that, the decay mechanism of positron emission initiates and coverts a proton into a neutron and emits positron. Then an annihilation process occurs in which the proton fuses with the adjacent electron which produces a pair of photons (γ rays). Each of two photons possess 511 keV energy and move in equal and opposite

directions. In the end, PET scanners, possessing γ-ray detectors, are present that detect photons when they collide with detectors on opposite sides of each other at the same interval of time. Figure 7 shows a PET/CT scanning device and the results obtained from it by Gauthé et al. (2015).

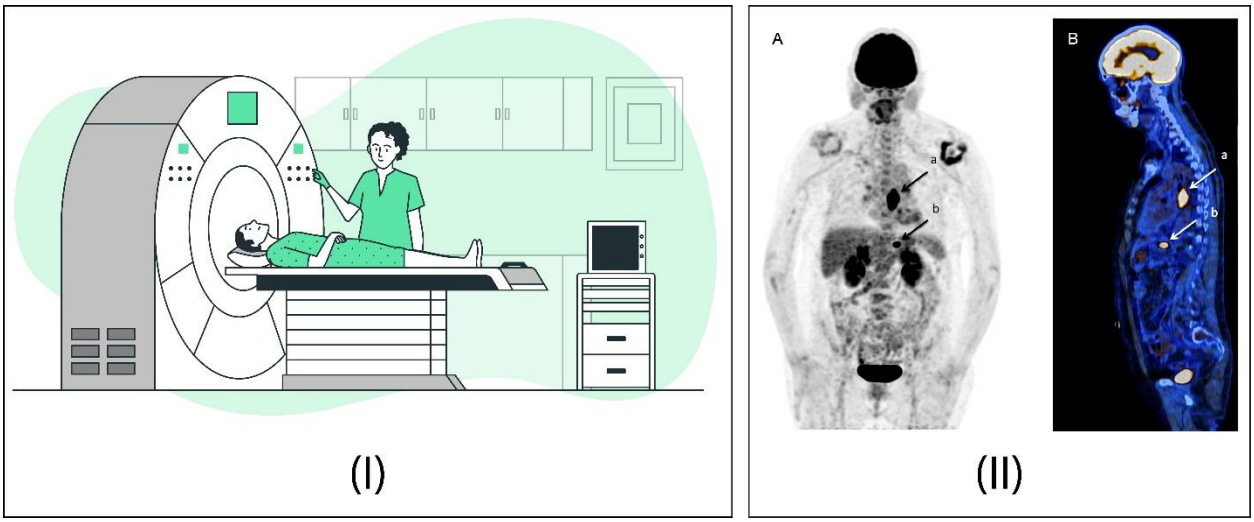

Figure 7: (I) A PET/CT scanning equipment (Designed by Freepik). (II) A PET/CT scan of a woman suffering from oesophageal squamous cell carcinoma. (A) PET/CT image, (B) showing the fluorodeoxyglucose uptake by oesophageal tumour (Figure 7 (II) is copied from Gauthé et al. (2015) under CC BY-NC-ND 4.0 License).

MRI is another non-invasive way of determining the internal structures and various characteristics of the functions inside the body. It is free from the risk related to exposure as it utilizes non-ionizing electromagnetic rays. In precisely regulated magnetic fields, radio frequency is applied to obtain high resolution cross-sectional graphics of the body in any dimension. To construct a MR image, the patient is placed inside a huge magnet which creates a strong external magnetic field. This field leads to the aligning of nuclei present in body with the magnetic field. Following this, with the utility of radio frequency signal, energy is discharged from the body, detected and utilized to create MR image via computer (Ravett 2011). Multiple MRI bioassays are well recognized and are used in clinical applications for the tumour detection. A few MRI biomarkers in applied in oncological studies are PI-RADS (Prostate Imaging-Reporting and Data System), LI-RADS and for the detection of prostate, liver and breast cancer, sequentially.

Another advanced bioimaging technique is radiomics. It comprises the acquisition of measurable parameters, that act as bioassays for structural alterations and abnormal physiological mechanisms in diseased conditions. Radiomics provides us with quantitative dataset that can be parsed, processed as well as analyzed by applying machine learning techniques (Gillies et al.,

2016). Radiomics-based bioassays offer valuable information for diagnosing, classifying and therapeutically managing multiple types of solid tumours. Radiomics has been reported to have significant role in the modulation of neurological cancer conditions such as brain metastases, glioblastoma multiforme and low-grade gliomas. Radiomics have extensive applications in the field of neuro-oncology, starting from the precise categorization of brain lesions (for instance, either lesions are metastases or gliomas), therapeutic planning as well as the evaluation of immunotherapy response (Shaikh et al., 2020).

7.2. Contribution of imaging biomarkers to cancer diagnosis:

Imaging biomarkers are easily accessible, economical and non-destructive tools that are used to screen and detect tumours as well as sequentially observe the patients i.e., evaluation of therapeutic response and recognition of therapy related problems. They can detect heterogeneity in tumours, screen the alterations in tumours over a course of time as well as determine the multiple lesions in a person (O'connor et al., 2017). Imaging of molecular mechanism has provided us the insight into the features of cancer, including proliferation, hypoxia, apoptosis, metabolism, angiogenesis, as well as determines the efficiency of targeted therapies.

Fluorodeoxyglucose is the frequently used radioisotope that measure metabolism of glucose. It is applied to detect and determine the stages of different types of cancers such as breast, colorectal and lung cancers (Gauthé et al., 2015). Imaging techniques, specifically breast imaging, have been extensively used for decades in biomarker-based studies and have proven to be effective. The optical imaging methods can be applied to determine the metabolic mechanisms at a cellular level. Fluorophores and reporter genes are used to graphically examine unique metabolic activities, i.e., ROS, activity of enzymes and pH. Optical imaging is the most frequently applied technique in experimental researches and preclinical studies. The metabolomic imaging methodologies provide insights into tumour metabolism and help in determining the biological features of tumour cells. Breast imaging methods and mammography are important for early diagnosis of breast cancer. Mammograms aid in detecting anomalies, i.e., masses or calcifications, that in turn helps us in early diagnosis and immediate treatment (Tang et al., 2009).

7.3. Challenges and future developments in imaging biomarker research

Although imaging biomarkers are non-invasive, widely available and technologically stable, but there are two major requirements to effectively apply them in clinical practice, that are ‘validation’ and ‘standardization’. Standardization is the measuring parameters for imaging and the methods applied after the formation of image. It is a crucial element to guarantee the

reproducibility of image in different locations and machinery. In order to meet this requirement, various consensus conferences have been organized to standardize diffusion MRI and perfusion MRI Comprehensive testing of fresh bioassays should be made for their reproducibility, precision, specificity and sensitivity. These tests should be carried out in models, animals as well as humans before their application in clinical medicine. The alterations in the levels of biomarkers should be associated to any biological effect and clinical effects. Precision medicine for cancer treatment is the most wanted target in oncology. The major problem that hindered the efficiency of biomarkers translation from discovery to employment was that the clinical translation of novel cancer bioassays remained sluggish. Despite multiple challenges, quantitative MRI markers are continually expanding to address the issues in personalized medicine for cancer patients, thanks to advancements in machine learning techniques (European Society of Radiology, 2010).

When a biomarker exhibits satisfactory biological, technical and clinical authentication, it must be qualified to be used for a certain purpose or use. The cost utility of bioassays is particularly crucial in resource-limited healthcare systems that prioritize outcome-focused care. Hence, the financial analysis is a key part of applying a new marker into routine practice. Therefore, the biomarkers should be prioritized that are cost-effective and precise. In the current era of artificial intelligence, the saturation of digital data is always increasing. So, the efforts should be increased in using validated and quantified imaging markers as the main approach to make therapy related decisions for patients (deSouza et al., 2019). Imaging biomarkers are of great importance in precision oncology. However, initiatives should be taken to make them common and prioritize them in clinical practice.

Conclusion

In conclusion, biomarker research has been integral to cancer treatment for many years. Multiple cancer biomarkers have been developed for the detection and treatment of cancer including CTCs, liquid biopsy, exosomes, ncRNAs, metabolomic markers and imaging bioassays. Although, these biomarkers were found to be effective against cancer and applied in the clinical practice. But there are many problems in the applications of these bioassays, such as, their sensitivity, stability and cost. Some recent studies where these bioassays were studied in combination and significant results were achieved for precision oncology. Therefore, the research should be conducted while integrating these biomarkers with each other to obtain better results. Nonetheless, the combined research and applications of these markers can be a promising innovation in the field of precision oncology and pave the way for precision medicine and targeted therapies.

Authors' Contribution

M.Z.S, G.M., T.H., and I.R. wrote the whole manuscript. I.R. and M.N.T. supervised the work and evaluated the whole manuscript. M.Z.S. and M.N.T. prepared the figures. All authors reviewed the whole manuscript.

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