Dichotomy of Urinary Proteins

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Urinary biomarkers offer non-invasive avenues for detecting cancers, potentially bypassing the invasiveness of biopsies. The investigation focuses primarily on breast and prostate cancers due to their prevalence among women and men, respectively. The intricate interplay of urinary proteins is explored, revealing a landscape where proteins exhibit context-dependent behaviors.

urinary proteins | breast cancer | prostate cancer | physical activities | surgery

1. Urinary Biomarkers for Breast Cancer and Prostate Cancer

It has been debatable whether screening breast cancer and prostate cancer does more harm than good [1][2], whereas precise screening and diagnostics are recommendable in determining the suitable course of cancer treatment [3]. Early detection not only facilitates timely decision making but also augments both the quality of life and the overall survival rate [4]. Leveraging urinary constituents as diagnostic indicators proves especially advantageous for cancers intricately tied to the urinary tract, encompassing urothelial carcinoma, bladder cancer, and non-muscle invasive bladder cancer [4][5][6][7]. Beyond this domain of urinary tract-related cancers, urinary markers have demonstrated their utility in detecting breast cancer and prostate cancer.

Breast cancer: Breast biopsies, a commonly employed method, entail invasiveness that can potentially expose patients to potential risks such as bruising, swelling, infection, and bleeding at the biopsy site [8]. In contrast, urine-based diagnostics present a non-invasive avenue [9]. A cluster of biomarkers is intertwined with the modulation of the extracellular matrix, exemplified by the matrix metalloproteinase 9 (MMP9) and neutrophil gelatinase-associated lipocalin (NGAL) complex [10].

Findings also indicate the significant elevation of a matrix metalloproteinase 1 (MMP1) and CD63 complex in the urine of breast cancer patients [11]. CD63, a cell surface molecule, binds with tissue inhibitor of metalloproteinases 1 (TIMP1). Furthermore, extracellular matrix protein 1 (ECM1), microtubule-associated serine/threonine kinase family member 4 (MAST4), and Filaggrin (FLG) exhibit heightened levels in the urine of breast cancer patients [12]. Apart from extracellular matrix proteinases, endothelial-derived gene 1 (EG1), expressed in both endothelial and epithelial cells, displays elevated levels not only in breast cancer but also in colon, prostate, and lung cancers [13]. Similarly, trefoil factor 1 (TFF1), a small secretory protein, demonstrates elevated levels across various cancer types, including breast cancer [14]. Moreover, ECM1, MAST4, FLG, and MAST4 are implicated as potential biomarkers for the preliminary indication of breast cancer presence [15].

Prostate cancer: At present, the serum prostate-specific antigen (PSA) stands as the foremost pivotal biomarker for discerning, tracking, and overseeing the treatment of prostate cancer [16][17][18][19][20]. Despite its instrumental role in substantially reducing prostate-cancer-related mortality, however, its utilization has also brought about the unintended consequences of excessive diagnosis and overtreatment of low-risk prostate cancer cases [21][22][23]. Consequently, an imperative exists for the development of more dependable, non-invasive approaches to prostate cancer diagnosis. Beyond PSA, another notable biomarker is prostate cancer antigen 3 (PCA3), marked by robust expression in individuals afflicted with prostate cancer [24]. This led to the FDA's 2012 approval of PCA3's use as a urine-based diagnostic tool for prostate cancer. Notably, the PCA3 level has been indicated to be independent of prostate size and serum PSA level [25][26][27]. Furthermore, within the urine of prostate cancer patients, the presence of Golgi membrane protein 1 (GOLM1) immunoreactivity has been identified, suggesting its potential as a biomarker for clinically localized prostate cancer [28].

In addition, Engrailed 2 protein (EN2), a transcription factor bearing a homeodomain, is secreted into the urine by prostate cancer cells, distinct from normal prostate tissue and benign prostatic hypertrophic cells that do not exhibit EN2 secretion [29]. The levels of urinary EN2 before radical prostatectomy have been linked to the stage of the tumor [30]. Another notable biomarker, TMPRSS2-ERG (V-ets erythroblastosis virus E26 oncogene homolog), fused with SAM-pointed domain-containing Ets-like factor (SPDEF), is recognized as a prostate-cancer-specific marker in urine [31][32]. Elevated SPDEF levels correlate with heightened aggressiveness and metastatic potential [13]. Furthermore, the urinary levels of β-2-microglobulin (β2M), pepsinogen A3 (PGA3), and mucin 3 (MUC3) were found to be elevated in prostate cancer patients [33]. Furthermore, urinary CD105 exhibited increased levels in men with biopsy-positive prostate cancer in comparison to those with biopsy-negative results [34]. The interleukin 18 binding protein (IL18BP), a potent inhibitor of IL-18, was also noted to be elevated in the urine of individuals with prostate cancer [12].

It is worth noting that urinary proteins are derived from blood filtration processes occurring in the kidneys. In cases where the filtration membrane of the kidneys is compromised by prostate cancer, it can result in the excretion of abnormal substances into the urine through this damaged filtration system. This condition, known as proteinuria, may arise due to various factors including kidney diseases and immune disorders [35][36].

2. Effects of Physical Activities

Physical activities have been known to be beneficial by strengthening bone and muscle and reducing inflammatory reactions, which are evidenced by serum proteome analyses [37][38][39][40][41]. Urine represents a filtrate of blood, thus rendering its protein constituents qualitatively akin to those present in the bloodstream. However, in comparison to serum proteins, urinary proteins tend to be more dilute and generally exhibit less complexity. Robust epidemiological evidence substantiates the protective impact of physical activity on breast cancer risk, recurrence, and mortality [42][43][44][45][46]. Research studies have elucidated that moderate exercise can distinctly enhance the prognosis of cancer patients by curbing tumor growth and forestalling metastasis [47][48][49][50][51]. Hydroxyproline serves as a prevalent urinary marker, indicative of the extent of connective tissue degradation encompassing bone, muscle, and other collagen and/or elastin-rich tissues [52][53][54]. Furthermore, research has indicated that physical

exercises, particularly aerobic modalities, bring about a reduction in urinary liver-type fatty acid binding protein (L-FABP) levels, as well as a decrease in urinary albumin excretion [55][56][57].

It is reported that mice bearing mammary tumors that had access to running wheels displayed diminished growth in both MCF-7 and MDA-MB-231 tumors [58][59][60]. In a prior study of ours, alterations in urinary proteins were observed by collecting samples from mice subjected to 5 min tibia loading, as well as from human individuals before and after a 30 min session of step aerobics. In comparison to urine samples collected before these loading activities or step aerobics, post-activity urine exhibited a reduction in cellular viability, proliferation, migration, and invasion of tumor cells in cell culture investigations [61]. After the activity, post-activity urine exhibited a significant increase in dopamine and melatonin levels while concurrently decreasing cholesterol, a compound associated with tumor promotion.

Prior research has established that dopamine and melatonin can downregulate Lrp5, a co-receptor involved in Wnt signaling pathways [55][56][57]. On the contrary, cholesterol is known to upregulate Lrp5, aligning with the observed effects of urine on Lrp5 expression. Furthermore, individuals conditioned by aerobic exercise displayed a substantial reduction in CD105 levels in their urine. CD105 is positioned downstream of Lrp5 and CSF1, with the latter being a hematopoietic growth factor linked to bone homeostasis and the progression of various cancers [57]. Additionally, CD105 is a component of the TGFβ receptor complex, and its role extends to tumor-associated angiogenesis [58]. These findings collectively contribute to the suppression of genes known to promote tumorigenesis, such as Snail, MMP9, Runx2, and PPARγ, within tumor cells. Additionally, administering diluted post-activity urine samples via intraperitoneal injection led to decreased tumor weight in the mammary fat pad within a mouse model of breast cancer [61]. These outcomes collectively underscore the potential of loading-conditioned urine not only as a prospective tumor suppressor but also as a wellspring of diagnostic biomarkers [62][63][64].

Aerobic exercise can also reduce the adverse effects of prolonged sitting, hypertension, and interstitial damage in patients with chronic kidney disease, by decreasing the level of urinary liver-type fatty acid binding protein (L-FABP) [55]. The level of urinary alkaline phosphatases is also reported to change in young men before and after 3 km running [65]. Similarly, swimming exercises can alter urine proteomics [66]. A study has been conducted to investigate the effects of long-distance cycling on specific urinary biomolecules [67]. The participants exhibited significant increases in the levels of serum lactate, uric acid, and bilirubin, even though they are not proteins. Notably, uric acid plays a crucial role in vascular regulation by increasing oxidative stress and promoting nitric oxide clearance, thereby inducing vasodilation [68]. An increase in bilirubin levels may contribute to a reduction in cardiovascular risk [69]. In summary, the growing body of evidence supports the beneficial impact of physical activity on both urinary proteins and non-protein biomolecules.

3. Treatment Effects

As surgical treatment affects urinary proteomes, two studies are reported for lumpectomy for breast cancer and prostatectomy for prostate cancer. When compared to healthy subjects, a noteworthy increase in the concentration

of urinary protein ADAM 12 was observed in patients with breast cancer who underwent lumpectomy. While the reported study cannot definitively establish a direct connection between the concentration of urinary protein ADAM 12 and the status and stage of breast cancer, it does suggest that surgical tumor resection has an impact on urinary protein [70]. Additionally, in the case of urine samples collected from patients with breast cancer following surgery or other treatments, a correlation has been established between urinary estrogen metabolism levels and breast cancer risk [71].

Regarding prostatectomy, distinct discrepancies in urinary proteomes were discerned between two groups of prostate cancer patients: those with positive surgical margins and those with negative margins. Notably, the positive margin group exhibited elevated levels of three proteins—cyclin-dependent kinase 6, galectin-3-binding protein, and L-lactate dehydrogenase C chain [72]. In a separate study, urine samples were procured from patients with prostate cancer and breast cancer, all of whom underwent external beam radiation therapy, without concurrent chemotherapy. The findings illuminated elevated levels of VEGF and MMP in patients with cancer compared to those in normal controls [73][74]. Furthermore, individuals with metastatic cancer displayed even higher VEGF and MMP levels when contrasted with patients diagnosed with non-metastatic cancer [73].

One of the primary objectives is to harness urinary proteins as a dual-purpose tool—both for diagnosis and as an indicator of the effectiveness of treatments like chemotherapy, radiotherapy, and surgery. A earlier research unveiled distinctive shifts in the urinary proteomes of prostate cancer patients, showcasing variable levels of tumor-modulating proteins in urine samples taken before and after prostatectomy. Initially, it is found that applying diluted urine obtained from patients after prostatectomy, a procedure entailing the surgical removal of the prostate led to a substantial reduction in the tumorigenic behaviors exhibited by prostate tumor cells. In post-prostatectomy urine, the levels of angiogenin [75][76]—a promoter of blood vessel formation—were significantly diminished [75]. Additionally, the post-prostatectomy urine demonstrated heightened concentrations of three cell-membrane proteins: PRSS8 [77], nectin 2 (PVRL2) [78], and NID1 [79]. Notably, these proteins exerted tumor-suppressive actions within the extracellular domain by inhibiting the expression of oncogenic genes such as Snail and TGFβ.

Of significant importance, NID1 emerges as a multifaceted protein with a dual role, whereby its functional impact varies based on its cellular location. In its extracellular milieu, NID1 exhibits tumor-suppressive qualities [80], while its intracellular presence potentiates tumor-promoting effects [81][82]. This dichotomy is notably demonstrated in the context of cell migration, where the introduction of recombinant NID1 or conditioned medium from cells overexpressing NID1 led to diminished migratory behavior in triple-negative breast cancer. These observations suggest that NID1's role as a tumor suppressor could potentially be harnessed as a valuable therapeutic avenue for the treatment of triple-negative breast cancer.

Utilizing quantitative-mass-spectrometry-based proteomic analysis, it was unveiled that NID1 proteins are secreted by endothelial cells, exerting an inhibitory effect on the migration of cancer cells induced by endothelial cells [80]. However, it is noteworthy that NID1's association with ovarian cancer reveals a contrasting facet. In this context, NID1 contributes to a poor prognosis by promoting invasion, migration, and chemoresistance in ovarian cancer through the activation of ERK/MAPK signaling [79]. On a related note, the role of the serine protease PRSS8

emerges as a potential suppressor in colorectal carcinogenesis and metastasis [83][84][85]. Its ectopic expression has demonstrated the capability to inhibit tumor growth both in vitro and in vivo, in addition to curbing the migration and invasion of non-small-cell lung cancer cells [86].

An advantage of using urine as a medium over blood is the relative stability of urinary proteins, as they do not undergo significant proteolysis within several hours of collection. Therefore, urinary proteomics offers an attractive avenue for the discovery of cancer biomarkers [87]. In the urinary proteomes of human prostate cancer specimens obtained after prostatectomy, variations have been observed between groups of patients with positive and negative surgical margins [72]. These differences can be linked to the underlying molecular mechanisms of prostate cancer development [72].

4. Double-Sided Role of Urinary Proteins

Not only urinary proteins but also some tumor suppressor proteins are double-sided and very dependent on the environment. Some oncogenic proteins in the cytoplasm and cell membrane are thought to promote tumor cell proliferation and migration but may conversely act as tumor suppressor proteins in the extracellular domain. For instance, extracellular Eno1 recombinant proteins are reported to suppress the metabolic activities of breast cancer cells and act as cytotoxic agents by downregulating Snail, TGFβ, and MMP9 [88]. By contrast, the overexpression of Eno1 in breast cancer cells upregulated the above tumorigenic genes and elevated their proliferation and transwell invasion [89]. The protein NID1 in urine and its recombinant protein reduced the EdU-based proliferation and scratch-based motility of TRAMP prostate cancer cells. In contrast, overexpression of NID1 stimulated the EdU-based proliferation and scratch-based motility of TRAMP cells. This shows that extracellular NID1 acts as a tumor suppressor gene, while intracellular NID1 acts as a tumor-promoting gene [75].

An intriguing question arises when considering the dual role of urinary proteins and their potential connection to proteoforms, which denote the diverse molecular forms of proteins. It remains uncertain whether a protein's functionality is influenced solely by distinct cellular locations, such as cytoplasmic and extracellular domains, or if variations in functions, like those in cell signaling, cell adhesion, and interactions, stem from modifications, cleavage, and other alterations. Addressing this question requires further studies to unravel the intricate relationships between urinary proteins, proteoforms, and their multifaceted roles. It is important to note that the double-sided role of urinary proteins may differ depending on not only their locations in the cytoplasm, extracellular space, or urine but also the distinctive microenvironments associated with cancer and interacting cells. The role in the urine can also be affected by age, diet, and hydration status. Given these complexities, further analyses are recommended to characterize their value as diagnostic and prognostic tools.

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