Aberrant Phosphorylation in Cancer

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Protein phosphorylation is a vital step for the coordination of cellular and molecular functions, such as the regulation of metabolism, proliferation, apoptosis, subcellular trafficking, inflammation, and other important physiological processes. Thus, altered expression of phosphoprotein will lead to deregulation of different signaling pathways as well as development and progression of cancer. Different expression patterns of various phosphoproteins have been reported in sera of various cancers and are focused.

biomarker phosphorylation dephosphorylation cancer

1. Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed malignancy worldwide, and the third most common cause of cancer-related death. HCC is associated with multiple risk factors and cofactors. In the majority (80–90%) of patients, HCC is preceded by cirrhosis. In this regard, chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is of particular concern. HBV-related chronic hepatitis is responsible for an estimated 50–80% of HCC cases worldwide, whereas 10–25% of cases may be due to HCV infection [1].

Despite existing serum diagnostic markers of HCC such as Alpha-fetoprotein (AFP), scientists have currently concentrated on characterization of phosphoprotein profiles in the serum of HCC patients to develop a precise early diagnostic biomarker of HCC. Hu et al. conducted a phosphoproteomics study on serum of 12 HCC patients and 12 healthy individuals [2]. Prior to MS analysis, phosphopeptide enrichment was performed using TiO_2 immobilized mesoporous silica particles. MALDI-TOF MS analysis initially identified four phosphopeptide peaks, indicating a potential difference between HCC patients and healthy individuals. The MS-based quantification of phosphopeptide, followed by a partial least-squares discriminate analysis (PLS-DA), revealed that the peptide D[pS]GEGDFLAEGGGV was upregulated, whereas the peptide of AD[pS]GEGDFLAEGGGVR was downregulated greatly. Minimal change was observed for the other two peptides, AD[pS]GEGDFLAEGGGV and D[pS]GEGDFLAEGGGVR. The result indicates that the first two phosphopeptides, D[pS]GEGDFLAEGGGV and AD[pS]GEGDFLAEGGGVR, may be effective diagnostic biomarkers for liver cancer [2]. There are several biomarkers that have the potential to dramatically improve the early detection of HCC.

2. Gallbladder Cancer

Gallbladder cancer (GBC) is a distinct type of biliary tract cancer that is rare, aggressive, and has limited treatment options apart from surgical resection, which has an estimated 5 year survival rate of 2% in metastatic disease. In

2022, an estimated 12,130 GBC and other biliary cancers are expected to be diagnosed, with an estimated 4400 patients dying from these diseases. Prognosis is particularly poor in elderly and racial minorities [3]. Tan et al. identified 24 differentially expressed proteins, which includes 12 upregulated and 12 downregulated proteins between gallbladder cancer patients and healthy controls [4]. Haptoglobin, S100A10, and other identified proteins may be potential molecular targets for early diagnostic and therapeutic application of GBC [4]. The immunohistochemistry showed that high expression of phosphorylated extracellular signal-regulated kinase 1(ERK1) at 202 threonine and ERK2 at 204 threonine residues in a gallbladder tumor was significantly associated with poor survival of gallbladder cancer patients [5].

3. Cholangiocarcinoma

Cholangiocarcinoma (CCA) is a highly malignant tumor arising from the epithelial cells lining the bile duct. The five-year survival rate for extrahepatic and intrahepatic bile duct cancer is 10% and 9%, respectively, whereas if they are diagnosed at an early stage, the 5-year survival rates are 17% and 25%. The slow progression makes it difficult for early diagnosis, and most cases are detected in advanced stages. Most of the approaches regarding diagnosis of CCA have focused on analysis of tumor tissue, biopsy, and proteomics of serum samples. In one of those studies, proteins FAM19A5, MAGED4B, KIAA0321, RBAK, and UPF3 were identified in the serum through a proteomic approach using highly stringent analysis with cross-validation [6]. The presence of these proteins can potentially discriminate patients with CCA from individuals having benign biliary tract diseases (BBTD). Kotawong et al. took a precise diagnostic approach for CCA by implementing phosphoproteomics on serum of 10 CCA patients, 5 *Opisthorchis viverrini* (OV) infected patients, and 5 healthy individuals [7]. Herein, 2D gel electrophoresis characterized 300 spots as phosphoproteins with two prominent 21 kDa upregulated spots, 21A and 21B. The LC-MS/MS analysis of the 21A and 21B spots revealed 98 and 64 identified proteins, respectively. Among these, MeV (Multiple Experiment Viewer) program-based bioinformatics and statistical analysis identified two proteins (trafficking protein particle complex subunit 5 and p115) which were significantly upregulated in plasma of CCA patients compared to the non CCA group. Those two above proteins are potential diagnostic biomarkers of CCA by combination of kinetic Monte Carlo (KMC) with a statistical *t*-test analysis [7].

4. Gastric Cancer

Gastric carcinoma (GC) is the fifth most common cancer and the fourth leading cause of cancer-related death worldwide in 2020. In the same year, an estimated 1.1 million cases (720,000 males and 370,000 females) of GC were diagnosed worldwide [8]. The serum phosphoproteomics study was also executed in GC patient samples to overcome the non-specific diagnosis of gastric cancer with existing carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) biomarkers. In order to understand the phosphopeptide profile in patients' serum, Zhai et al. initially conducted a phosphoproteomics profile on 20 GC patients and 20 healthy individuals by MALDI-TOF MS analysis [9]. After phosphopeptide enrichment with TiO_2, the mass spectrum of the serum sample initially showed four remarkable ion peaks. Subsequently, tandem mass spectrometry (MS/MS) characterized four ion peaks corresponding to phosphopeptides, ADpSGEGDFLAEGGGV (F1), DpSGEGDFLAEGGGV (F2), DpSGEGDFLAEGGGVR(F3) and ADpSGEGDFLAEGGGVR (F4) at m/z 1389.3, 1460.4, 1545.5, and 1616.6, respectively, which were all derived from fibrinopeptides A. Further, absolute quantification of the four endogenous phosphopeptides in serum by MS revealed the F3 phosphopeptide was significantly downregulated in GC samples compared to healthy controls. They extended their study on a training set of 40 GC patients and 30 healthy individuals, and a validation set of 20 GC patients and 30 healthy individuals, in order to establish serum phosphopeptides as potential diagnostic biomarkers of GC [10]. Interestingly, the F3 level in sera was significantly reduced in eight out of nine patients (89%) at stage I. When F3 was incorporated into receiver operating characteristic (ROC) analysis independently, GC could be distinguished from controls with 88.3% specificity and 96.7% sensitivity. This suggested evaluation of F3 phosphopeptide level from fibrinopeptides A of fibrinogen in serum of GC patients could be used as potential early diagnostic marker of GC [10].

5. Lung Cancer

Lung cancer (LC) is the most common malignancy worldwide and is the leading cause of death. There were more than 2.2 million new cases of lung cancer in 2020. In India, lung cancer constitutes 6.9% of all new cancer cases, and lung cancer-related death accounts for 9.3% of all cancer-related death. Small cell lung cancer (SCLC) accounts for <20% of LC cases, whereas non-small cell lung carcinoma (NSCLC) cases are a majority, with adenocarcinoma (ADC) 32%, squamous cell carcinoma (SCC) 30%, and large cell carcinoma (LCC) 10%. Despite the progress in LC research and advancement in treatment strategies, the five-year survival rate for patients with LC was <15%. Poor prognosis is mainly attributed to late diagnosis, with the majority of LC patients diagnosed at an advanced stage when the surgical resection is hardly possible. Tremendous amounts of evidence suggest that genetic abnormalities contribute to the development of lung cancer. These molecular abnormalities may serve as diagnostic, prognostic, and predictive biomarkers for this deadly disease.

6. Prostate Cancer

Prostate Cancer (PCa) is the second most commonly diagnosed cancer, and the fifth leading cause of cancer-related death among men worldwide, with an estimated 1,414,000 new cancer cases and 375,304 deaths in 2020 [11]. The serum prostate specific antigen (PSA) test has been the leading method of screening for prostate cancer. The PSA test, in conjunction with other common tests like digital rectal examination (DRE) or transrectal ultrasound (TRUS), can reveal the probability of the incidence of prostate cancer [12]. Based on the report from the National Prostate Cancer Detection Project of the American Cancer Society, 92% of cancers detected by PSA, DRE, and TRUS from an annual testing are localized to the prostate [13]. However, the treatment options for all localized prostate cancer are not the same, as some prostate tumors are aggressive and some are indolent. Unfortunately, the current clinical biomarkers for prostate cancer are not ideal to specifically distinguish between those patients who should be treated adequately to stop the aggressive form of the disease and those who should avoid overtreatment of the indolent form. To search for the reliable biomarkers of prostate cancer, Liu et al. assessed 80 serum samples from four groups: men without PCa, patients with low risk primary PCa, patients with high risk primary PCa, and patients with metastatic PCa (n = 20 per group) [14]. Among the target proteins, nine of them

(PTN, MK, PVRL4, EPHA2, TFPI-2, hK11, SYND1, ANGPT2, and hK14) were found to be significantly increased in the metastatic PCa group compared to others. In another study, six (CASP8, MSLN, FGFBP1, ICOSLG, TIE2, and S100A4) out of 174 target proteins were found to be significantly decreased after radical prostatectomy (RP) in patient-matched serum samples from ten men with high grade and high-volume prostate cancer [15]. Tony et al. identified and evaluated a potential serum protein signature of disease recurrence in a cohort of PCa patients that received treatment with combined hormone and radiation therapy (CHRT) [16].

7. Breast Cancer

Several studies reported the association of altered expression of serum proteins and changes in their glycosylation pattern and miRNA with the pathogenesis of breast cancer. These studies were conducted to find biomarkers for early diagnosis of the disease [17][18]. However, the potential use of serum phosphoproteins as biomarkers for diagnosis and prognosis of breast cancer was limited. Chen et al. [19] isolated and identified phosphoproteins in extracellular vesicles (EV) from human plasma as potential biomarkers to discriminate breast cancer patients from a healthy control. Eighteen breast cancer patients and six healthy individuals were included in their study. Phosphopeptides generated from the EV were enriched and analyzed by LC-MS/MS. They quantified 3607 and 461 unique phosphosites and identified 156 and 271 phosphosites with significant changes in microvesicles and exosomes, respectively. They compared these phosphosites representing 197 unique phosphopeptides that showed a significant increase in patients with breast cancer with all identified unique phosphopeptides in EV phosphoproteomes. They also found that a significant portion of these 197 phosphopeptides (>60%) were also identified by the proteogenomic study, which indicates that EV phosphoproteome was sensitive and could be used to identify phosphorylation events that were disease-specific [19]. In another study, protein profiling using antibody microarrays with 215 highly specific pre-selected antibodies was designed for different proteins and specific phosphorylation sites. Results indicate that p-S259 JunB, p-S79 JunB, and p-T512 ICAM-1 phosphoproteins were significantly upregulated in the plasma of breast cancer patients compared to healthy individuals [20]. This predicts that phosphoproteins could be a candidate for early diagnosis of breast cancer marker.

8. Colorectal Cancer

Annually, around 10% of cancer-related deaths are due to colorectal cancer (CRC) [21][22][23]. It has been observed that the incidence rates are rising in developing countries [21]. Males are at a higher risk for developing colorectal carcinoma compared to females. In 2020, the global CRC incidence rate in men (23.4 cases per 100,000 persons) was 44% higher than that in women (16.2 cases per 100,000 persons) [21].

9. Pancreatic Cancer

Pancreatic cancer (PaCa) ranks fourteenth among all the cancers worldwide as per GLOBOCAN 2018, with varying incidence rates across the globe. In India, there is also regional variation in the incidence across the country, with the northeast showing the highest rates [24]. Like cholangiocarcinoma, the five-year disease-free survival rate of PaCa is very low (~5%), as there was no sensitive and specific early diagnostic biomarker [25]. Some studies suggested a few serous proteins as specific biomarkers of PaCa, but translating these results into cost-effective and reliable clinical tests is very difficult [26][27]. Takano et al. attempted to observe the expression of circulating serum phosphoproteins in serum from 26 PaCa patients and 25 healthy individuals training set followed by validation set 1 and 2 to establish the impact of serum phosphoprotein as early diagnostic markers of PaCa. Herein, a Bio-Plex immunoassay was performed and revealed six phosphoproteins which showed significantly increased expression in serum from PaCa patients compared to healthy individuals. Among these, p-T202 ERK1, p-S201 ERK2, and p-T44 MEK1 proteins primarily proposed as potential diagnostic markers of PaCa. These phosphoproteins were found to be correlated with serum and tissue biopsy. However, the sensitivity of serum p-ERK1 and p-ERK2 was found to be better for prediction of stage-I PaCa than CA19-9, indicating that serum p-ERK1 and ERK2 could be a potential early diagnostic marker of PaCa [28].

10. Renal Cell Carcinoma

The highest prevalence of renal cell carcinoma (RCC) was accounted in the western countries and approximately accounts for 3% of all types of cancers. RCC patients without any metastasis showed a 93% five-year survival rate. However, with diagnosis at the advanced stage, the 5-year survival rate is 71% and is reduced to 14% when metastasis occurs. Ljungberg et al. reported a 2% annual increase in occurrence of RCC worldwide [29].

Diagnosis of RCC by biopsy is an invasive, painful, and complex method that often requires an expert radiologist in CT or USG. Instead of an invasive kidney biopsy, a simpler and non-invasive method such as using different miRNA, proteins, and phosphoproteins as biomarkers of kidney cancer has been considered. Recently, a phosphoproteomics study was conducted to distinguish kidney cancer accurately from non-cancerous conditions. Phosphoproteins extracted from extracellular vesicles and isolated from serum samples of chronic kidney disease (CKD), RCC patients, and from healthy controls were analyzed by LC-MS. LC-MS investigation with phosphopeptide enriched sample revealed 146 phosphoproteins which showed significant change in kidney cancer samples compared to the control. Similarly, 156 phosphoproteins were characterized, which showed a sharp change in CKD samples compared to control. Comparison between RCC and CKD samples revealed 44 phosphoproteins that were significantly different between these groups. Statistical analyses in the same set of samples revealed p-S107, S185 CRK-like protein (CRKL), and p-S298, S426 LYRIC (MTDH) phosphoproteins, which were found to be significantly high in abundance in RCC samples compared to CKD and control.

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