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Review Article: Blood Proteins as Potent Biomarkers for Colorectal Carcinoma

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ABSTRACT

Colorectal cancer (CRC) is the 3rd most frequent epithelial tumor globally which affect millions of people. Since this tumor transformed slowly from removable precancerous lesions, lesion diagnosing at an early stage can decrease the incidence of malignancy related mortality. Although colonoscopy markedly improves CRC detection rate, it is inconvenient and expensive. Thus, novel non-invasive biomarkers are needed to enable us to early detect CRC. Colorectal carcinoma is a complicated process including several changes in proteomic and genomic levels. From another hand, easy clinical samples particularly blood samples contain many secretory proteins. These up regulated proteins enhanced carcinogenesis, including cancer cells adhesion, migration and invasion. Continuing intense study in this research area promises emergence of novel superior non-invasive CRC screening markers that will allow the development of improved CRC prevention strategies. This review focuses on different secretory protein CRC biomarkers.

Keywords: CRC, Diagnosis, Prognosis, Circulating-Proteins, Marker.

1. INTRODUCTION

Worldwide, colorectal cancer (CRC) is the 3rd most commonly occurring tumor, representing, in 2020, about 10% of all newly diagnosed tumors [1]. Also, it is the 2nd most deadly malignancy. In 2020, there was an estimated 1.93 million newly diagnosed CRC patients, and 0.94 million CRC related-deaths globally [1].

CRC is a complicated process including several changes in proteomic and genomic levels [2]. It progresses gradually from benign conditions (polyps and cysts) to metastases and an advanced stage [3]. At CRC diagnosis, 20 and 36% of all cases present distant metastatic and regional spread lesions,

respectively [4]. Moreover, 50% of cases diagnosed with localized CRC will progress on to metastatic CRC [4]. CRC progression from a localized disease to a late metastatic tumor is usually indicative of patients' poor prognosis, as the 5-year survival rate reduces rapidly to 10% compared to 90% in early-stage tumors [4].

Despite several clinical reports suggested that it is important to monitor cell adhesion proteins that have a role in controlling further tumor metastasis and invasion [5]. Biomolecules like protein are the most available biological substances and biomarkers determination in cases with chronic disorders and malignancies including CRC could be of extreme clinical importance, because cancer cells produce numerous proteins in the blood stream and tissues [6]. When compared to healthy individuals, some literatures state that cancers produce several proteins in the blood [3, 7].

Owing to its complex function and structure, proteome study is still specifically challenging [8]. Clinical specimens like tissues, stool and particularly blood contain many secretory proteins [3]. These proteins are important for normal cell signaling and mechanisms, they are released by varied body tissues and organs [9]. Some proteins are particularly produced during pathological conditions, it's basically because of the chromosomal genetic variations and alternative mRNA synthesis [10]. Thus regarding pathological conditions of the tumor, the wide active range of protein has been elevated and considered as new biomarkers to evaluate the cancer status [3]. Eventually, proteomic reports identified a number of proteins that are benchmarked for CRC therapeutics and identification [10]. For example, glycoprotein over expression is needed for cancer development, growth and metastasis [11]. Released blood proteins are multifunctional and are involved in many tumors such as liver, prostate, lung, stomach, CRC, etc [12]. Moreover, these over-expressed proteins enhanced carcinogenesis, involving cancer cells adhesion, migration and invasion [13].

CRC biomarkers may be immunological, metabolites, epigenetic, genetic and protein biomarkers [3, 14, 15]. In this review, we regarded the several secretory protein CRC biomarkers.

2. MATERIALS AND METHODS

Protein marker identification has been performed in three different phases (Figure 1). Initially, the identified proteins are subjected to verification, development and finally clinically validated (Fig. 1) [16]. Protein biomarkers can be detected by several tools such as high- performance microarrays, radiological assays, 2-dimensional electrophoresis, mass spectrometry and western blot (Fig. 2) [17]. Cancer molecular study is basically depends on evaluating protein expression level and its related prognostic findings to help in taking clinical decision for tumor therapy [18]. Mostly in clinical diagnosis, protein biomarkers are used for over-expressed proteins assessments in clinical specimens which is performed by varied immunoassay approaches such as immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), and so on [19].

Fig (1): Pipeline showed phases of protein biomarker discovery

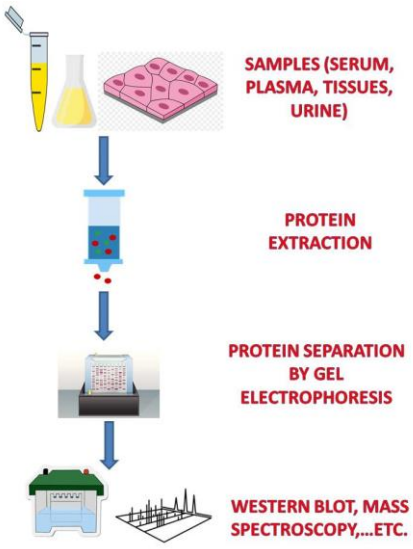

Fig (2): Flowchart showed proteomic tools for protein marker analysis

3. Carbohydrate Antigen (CA19-9) and Carcinoembryonic antigen (CEA)

To early detect CRC, physicians use a strong follow-up program that includes clinical examination and blood tests, involving searching for biological cancer biomarkers such as CEA and CA19-9 [20]. CEA is an oncofetal tumor marker discovered by Gold and Freedman in 1965 [21]. In the diagnosis of CRC, CEA is significant in 70% of cases. Well-differentiated adenocarcinoma is associated with elevated CEA serum levels. For a period of few months post-surgery, its increase is speaking in favor of tumor recurrence. Its level is also related to CRC size. So, smaller-sized tumors have normal serum CEA levels, and only tumors >3 cm are accompanied by high CEA level [22-24]. Also, cancer antigen CA19-9 elevated serum level is detected in case of CRC. It is a tumor marker that is reported to be increased in serum of cases with metastatic CRC [24, 25]. Several studies reported sensitivity of 26-48% for CA19-9 and 65-74% for CEA in CRC patients [26-28]. In contrast to CA19-9 low sensitivity, reports demonstrated that CA19-9 is correlated with CEA and, thus, enhances its sensitivity [29-32]. These tumor-associated antigens are membrane-anchored glycoproteins and are overexpressed in the majority of primary CRCs and also the metastatic CRCs. Thus, as reported by recent researches, they constitute promising target molecules for CRC treatments. Therefore, several monoclonal antibodies targeted these molecules have been developed with therapeutic proposes [33].

4. Heat shock proteins

Under stressful conditions like oxidative stress or hyperthermia starvation, normal cells synthesize some molecular chaperones called heat shock proteins (HSPs) as defense mechanism [34]. The tumor cells are binds to chaperones and stimulate its expression to promote abnormal cell growth and prevent cell apoptosis [35, 36]. Based on their molecular weight, HSPs are classified into different groups such as HSP110, HSP90, HSP70, HSP60, HSP40 and HSP10 [3]. HSPs overexpression has been extensively associated with CRC severity by regulating varied cell growth-related molecules and pathways in CRC cells [37-39]. They are represented as cancer development regulators, and reports have greatly demonstrated their utility as CRC markers for progression and detection [40]. Moreover in CRC, their elevated expression could be a good predictive biomarker for lymph node invasion [40, 41]. As depicted in Fig. 3, the overexpression of these HSPs has multiple oncogenic roles [42]. For diagnosing CRC, although the specificity was reported to be as high as >90%, the sensitivity of these proteins was reported to be low (40%) in some studies. Specificity and sensitivity of these proteins are impacted by the used assay analytical performance [43, 44].

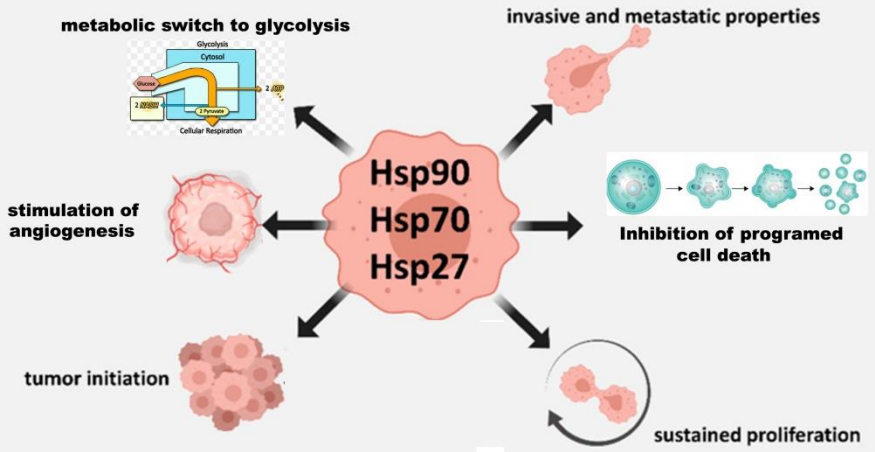

Fig (3): Cancer cell intrinsic mechanisms of tumorigenesis mediated by HSPs

5. Enzymes

Enzymes as biological markers are very strong approach for monitoring cell functions progression. To manage chronic diseases including tumors, they effectively show a new treatment way [3]. Telomerase is ribonucleoprotein complex that includes transcriptional process for genome integrity and DNA synthesis. Telomere length maintenance by telomerase is important to preserve cancer cells replicative potential [45]. Despite that telomeres are shorter in CRC compared to adjacent mucosa, there is general agreement that telomerase activity and/or expression levels are greater in CRC than in adjacent non-cancerous mucosa [46-48]. Studies demonstrate that cell-free circulating telomerase mRNA is a potential CRC biomarker [49].

On the other hand, antioxidant enzymes such as glutathione-S-transferase (GST) [50], arginine methyltransferase 1 [51], glutathione reductase (GRx) [52], catalase (CAT) [53], and superoxide dismutase (SOD) [54] are considered as plasma prognostic biomarkers for CRC risk.

6. Tissue inhibitor of matrix metalloproteinase 1 (TIMP-1)

Tumor cells act toward neighbouring cells via extracellular matrix (ECM) and a number of receptors [55]. Normal protein function and matrix metalloproteinases (MMPs) significantly alter the connections between surrounding cells and the ECM. ECM degrading mechanism based mainly on MMPs and their activities have been modulated by the TIMPs [56]. With varying conditions, these TIMPs can stop different MMPs functions. Compared to their normal counterparts, human CRC stimulates TIMP-1 synthesis in cancer models, eventually increasing tumors growth. Furthermore, in colon cancer, TIMP-1 and cancer-associated fibroblasts (CAFs) accumulation regulates CRC progression [3, 57]. In colon cancer, TIMP-1 has growth promoting properties thus stimulates cancer growth by inhibiting apoptosis [58]. In a meta-analysis evaluated the diagnostic role of TIMP-1 as serum biomarker for CRC diagnosis, the pooled analysis of all included studies revealed a specificity of 0.87 and a sensitivity of 0.65 [59].

7. Chemokines

Chemokines are a group of structurally related small peptides implicated in several events such as development of lymphoid tissue and angiogenesis [60]. However, in many pathological processes including autoimmune diseases, HIV infection, atherosclerosis, and even cancer, chemokines play also an important role. Through different mechanisms, it was demonstrated that some chemokines may involve in cancer development and metastasis [61, 62]. One of CRC key risk factors is chronic inflammation, so it was demonstrated that pro-inflammatory chemokines may be implicated in CRC progression, development, and invasion [63, 64]. The most frequent site of CRC metastasis is the liver, in which,

CXCL12 was reported to be normally secreted by endothelial and Kupffer cells [65]. Also, CRC cells can express this chemokine ligand [66]. Some studies reported that CXCR4 expression in CRC is associated with liver metastasis and poor survival rates [67, 68]. In addition, in CRC cells, CXCR7 is another receptor that was found to be expressed and interacts with CXCL12 [69]. Expression of CXCR7, CXCR4, and CXCL12 was assessed in CRC with lung invasion and results revealed that CXCR7 and CXCL12 expression was markedly greater in lung tissue samples compared to patients with primary lesions [62, 69]. As shown in Table 1, other chemokines and their particular receptors are implicated in CRC.

Table 1. Chemokines and their receptors in colorectal cancer [62].

Chemokines subfamily	Chemokines	Alters in colorectal cancer
CC (β) subfamily	CCL20 (LARC, MIP-3a)	Increased expression in CRC with hepatic invasion
CXC (α) subfamily	CXCL5 (ENA-78)	Elevated serum concentrations in CRC
	CXCL10 (IP-10)	CRC recurrence prognostic factor
	CXCL9 (Mig)	Elevated expression in CRC
	CXCL15	Elevated serum concentrations in CRC
	CXCL12 (SDF-1)	Increased expression in CRC and correlated with poor outcomes
	CXCL8 (IL-8)	Increased serum levels in CRC and distant metastases
CXCR1, CXCR2		Increased expression in CRC and metastases
CXCR3		Expression stimulates lymph nodes invasion
CXCR4, CXCR7		Association with liver invasion and poor prognosis
CCR6		Increased expression in liver invasion and CRC

CXCR, CXC chemokine receptor; CXCL, CXC motif chemokine ligand; IL, interleukin; MIP-3a, macrophage inflammatory protein-3a. LARC, liver and activation-regulated chemokine; CRC, colorectal cancer

8. **Cyclin D1**

In cancers as an oncogenic driver, cyclin D1 is mainly reported in different tumors, and the inhibition of cyclin D1/cyclin-dependent kinase (CDK) 4/6 axis represents an interesting target for tumor treatment [70]. In CRCs, former cyclin D1 immunohistochemical studies have demonstrated its prognostic utility, but with conflicting findings [71]. Generally, the overexpression of cyclin D1 in CRC can be used as a potential prognostic biomarker. Also, this expression may be serious in predicting responses to CDK4/6 inhibitors [72]. In CRCs, CDK4/6 inhibitors' therapeutic potential was assessed in combination with other agents, like mitogen-activated protein kinase (MAPK) inhibitors and immune checkpoint, Raf, [73]. KRAS-mutant CRCs have been reported to be specifically sensitive to a combination of CDK4/6 inhibitors and MAPK [74]. Some studies reported high sensitivity (95%) and specificity (95%) in CRC detection [75].

9. **S100P**

Among other metastasis drivers, S100P (S100 calcium-binding protein P) has been found to elevate cell motility and proliferation, and so the progression of many solid tumors [76, 77]. In rat mammary cell

lines, its overexpression has been reported to promote metastasis of benign cells [78], and further studies link S100P mechanistically to increased collective cell invasion, as well as individual cell migration [77]. This protein serves as a prognostic marker for gastrointestinal tumors, including CRC [77, 79].

10. Cytokines

For their development and growth, malignant cancers have the ability to create a permissive microenvironment and remodel its structure [80]. Consecutively, cancer cells release soluble factors like proteases, growth factors and cytokines. These factors stimulate cancer cell differentiation, growth, survival and progression [81]. CRC cells induce macrophage to produce IL-1β. NF-κB activation via IL-1β is coupled to the Wnt signaling stimulation and glycogen synthase kinase-3 beta (GSK3β) function inactivation in CRC cells [82]. Human CRC are infiltrated by inflammatory cells such as macrophages and mast cells, which release TNFα. In tumor-bearing mice as animal model, TNFα is elevated and mast cells were markedly depleted. TNFα decreased levels, indicated that mast cells are important TNFα sources. Predominantly in colon cancer, anti-TNFα treatment or mast cell depletion markedly suppressed polys [3]. Compared to cases without metastasis, it was reported that serum IL-6 elevated concentrations were related to metastatic stage of CRC [83]. Moreover, IL-17 enhance cancer cell to produce several angiogenic factors, including macrophage inflammatory protein-2, keratinocyte-derived chemokine PGE1, PGE2, and VEGF which promote angiogenesis in cancer [3].

11. Defensin alpha (DEFA)

The association between malignant tumors and DEFAs has been partially studied. DEFAs were found to be closely related to CRC [84, 85]. In the tissues and serum of CRC, DEFAs 1-3 were reported to be increased. Compared to normal epithelium, DEFA1-3 tissues and serum expression levels are higher in colorectal adenomas. Its sensitivity level in diagnosing CRC in patients' serum was 69%, and the specificity was 100% [86]. DEFA1–3 expression levels were related to hepatic- or lymphatic- metastasis [87]. DEFA5 may be a favorable prognostic factor and was reported to inhibit CRC progression [88]. In CRC formation, DEFA5 and DEFA6 are considered key factors [85, 89]. Microarray expression data analysis performed on 283 normal and cancerous tissues showed that DEFA6 was maximally expressed in CRC [90]. By suppressing DEFA6 function by shRNA, DEFA6 promoted the colony-forming ability, invasion, migration and proliferation of CRC cells [85].

12. Future prospective of protein CRC biomarkers

Molecular marker identification specifically receptor/secretory proteins could enhance and improve treatment strategies in CRC [18]. Marker development may result in targeting tumors treatments and improves adjuvant selection for drug development [91]. Unfortunately, most CRC patients are diagnosed at advanced stages so an inexpensive, non-invasive and automated standardized protein biomarker is needed for early CRC detection [92]. Moreover, protein markers use might reduce the economic burden in tumor therapy [93]. Advancement is required in the available CRC marker screening methods with high efficiency. More specific tissue and serum proteins need to be identified and evaluated in the CRC cases and it may improve novel drug development.

13. CONCLUSION

We conclude that tissue and serum protein markers should be followed up for CRC early detection. Also, these proteins can markedly manage CRC progression.

Conflict of interest: There is no conflict of interest.

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