Alfarama Journal of Basic & Applied Sciences

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January 2025, Volume 6, Issue I

DOI: 10.21608/AJBAS.2024.327588.1235

ISSN 2682-275X

Submitted: 12/10/2024
Accepted: 18/12/2024

Pages: 39 - 52

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

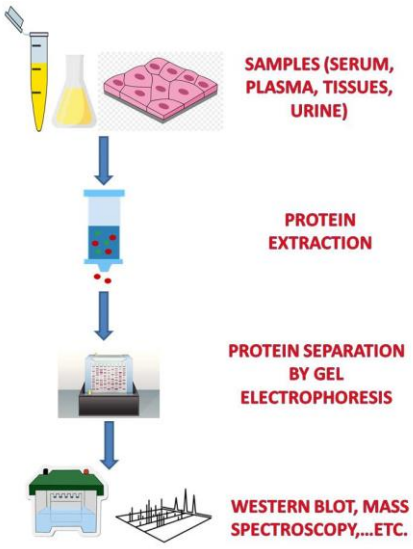

Fig (2): Flowchart showed proteomic tools for protein marker analysis

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].

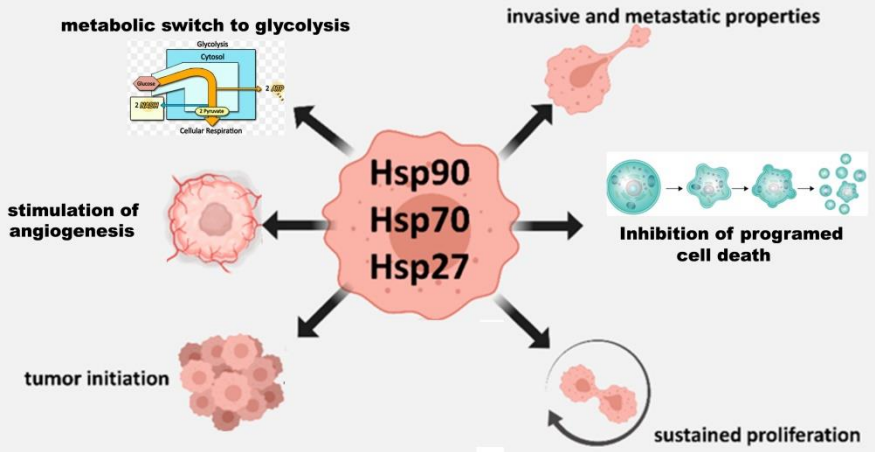

Fig (3): Cancer cell intrinsic mechanisms of tumorigenesis mediated by HSPs

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.

14. REFERENCES

[1] Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 2021;14(10):101174. doi: 10.1016/j.tranon.2021.101174

[2] Tanaka A, Ogawa M, Zhou Y, Namba K, Hendrickson RC, Miele MM, et al. Proteogenomic characterization of primary colorectal cancer and metastatic progression identifies proteome-based subtypes and signatures. Cell Rep. 2024;43(2):113810. doi: 10.1016/j.celrep.2024.113810

[3] Kuppusamy P, Govindan N, Yusoff MM, Ichwan SJA. Proteins are potent biomarkers to detect colon cancer progression. Saudi J Biol Sci. 2017;24(6):1212-1221. doi: 10.1016/j.sjbs.2014.09.017

[4] Thng DKH, Hooi L, Siew BE, Lee KY, Tan IJ, Lieske B, et al. A functional personalised oncology approach against metastatic colorectal cancer in matched patient derived organoids. NPJ Precis Oncol. 2024;8(1):52. doi: 10.1038/s41698-024-00543-8

[5] Janiszewska M, Primi MC, Izard T. Cell adhesion in cancer: Beyond the migration of single cells. J Biol Chem. 2020;295(8):2495-2505. doi: 10.1074/jbc.REV119.007759

[6] Martín-García D, García-Aranda M, Redondo M. Biomarker Identification through Proteomics in Colorectal Cancer. Int J Mol Sci. 2024;25(4):doi: 10.3390/ijms25042283

[7] Fijneman RJ, de Wit M, Pourghiasian M, Piersma SR, Pham TV, Warmoes MO, et al. Proximal fluid proteome profiling of mouse colon tumors reveals biomarkers for early diagnosis of human colorectal cancer. Clin Cancer Res. 2012;18(9):2613-24. doi: 10.1158/1078-0432.Ccr-11-1937

[8] Huff-Lonergan E ,Lonergan SM. Proteomics approaches – their potential for answering complex questions in meat science research. Italian J Animal Sci. 2023;22(1):911-924. doi: 10.1080/1828051X.2023.2248182

[9] Su J, Song Y, Zhu Z, Huang X, Fan J, Qiao J, et al. Cell-cell communication: new insights and clinical implications. Signal Transduct Target Ther. 2024;9(1):196. doi: 10.1038/s41392-024-01888-z

[10] Ogunwobi OO, Mahmood F, Akingboye A. Biomarkers in Colorectal Cancer: Current Research and Future Prospects. Int J Mol Sci. 2020;21(15):doi: 10.3390/ijms21155311

[11] Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, Jaymand M, et al. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun Signal. 2020;18(1):59. doi: 10.1186/s12964-020-0530-4

[12] Zhou Y, Tao L, Qiu J, Xu J, Yang X, Zhang Y, et al. Tumor biomarkers for diagnosis, prognosis and targeted therapy. Signal Transduct Target Ther. 2024;9(1):132. doi: 10.1038/s41392-024-01823-2

[13] Yuan Z, Li Y, Zhang S, Wang X, Dou H, Yu X, et al. Extracellular matrix remodeling in tumor progression and immune escape: from mechanisms to treatments. Mol Cancer. 2023;22(1):48. doi: 10.1186/s12943-023-01744-8

[14] Islam MS, Gopalan V, Lam AK, Shiddiky MJA. Current advances in detecting genetic and epigenetic biomarkers of colorectal cancer. Biosens Bioelectron. 2023;239(115611. doi: 10.1016/j.bios.2023.115611

[15] Órdenes P, Carril Pardo C, Elizondo-Vega R, Oyarce K. Current Research on Molecular Biomarkers for Colorectal Cancer in Stool Samples. Biology (Basel). 2023;13(1):doi: 10.3390/biology13010015

[16] Watts-Oquendo E, Sánchez-Peña M, Isaza CE, Cabrera-Ríos M. Potential colon cancer biomarker search using more than two performance measures in a multiple criteria optimization approach. P R Health Sci J. 2012;31(2):59-63.

[17] Al-Amrani S, Al-Jabri Z, Al-Zaabi A, Alshekaili J, Al-Khabori M. Proteomics: Concepts and applications in human medicine. World J Biol Chem. 2021;12(5):57-69. doi: 10.4331/wjbc.v12.i5.57

[18] Das S, Dey MK, Devireddy R, Gartia MR. Biomarkers in Cancer Detection, Diagnosis, and Prognosis. Sensors (Basel). 2023;24(1):doi: 10.3390/s24010037

[19] Lengfeld J, Zhang H, Stoesz S, Murali R, Pass F, Greene MI, et al. Challenges in Detection of Serum Oncoprotein: Relevance to Breast Cancer Diagnostics. Breast Cancer (Dove Med Press). 2021;13(575-593. doi: 10.2147/bctt.S331844

[20] Alragig M, Ermiah E, Gaber M, Algouti M, Rabie A, Jebri A, et al. Prognostic Value of Carcinoembryonic Antigen (CEA) and Carbohydrate Antigen (CA19-9) in Colorectal Cancer. Libyan International Journal of Oncology. 2024;46-58.

[21] Gold P ,Freedman SO. Demonstration Of Tumor-Specific Antigens In Human Colonic Carcinomata By Immunological Tolerance And Absorption Techniques. J Exp Med. 1965;121(3):439-62. doi: 10.1084/jem.121.3.439

[22] Kannagi R, Izawa M, Koike T, Miyazaki K, Kimura N. Carbohydrate-mediated cell adhesion in cancer metastasis and angiogenesis. Cancer Sci. 2004;95(5):377-84. doi: 10.1111/j.1349-7006.2004.tb03219.x

[23] Lakemeyer L, Sander S, Wittau M, Henne-Bruns D, Kornmann M, Lemke J. Diagnostic and Prognostic Value of CEA and CA19-9 in Colorectal Cancer. Diseases. 2021;9(1):doi: 10.3390/diseases9010021

[24] Vukobrat-Bijedic Z, Husic-Selimovic A, Sofic A, Bijedic N, Bjelogrlic I, Gogov B, et al. Cancer Antigens (CEA and CA 19-9) as Markers of Advanced Stage of Colorectal Carcinoma. Med Arch. 2013;67(6):397-401. doi: 10.5455/medarh.2013.67.397-401

[25] Lee T, Teng TZJ, Shelat VG. Carbohydrate antigen 19-9 - tumor marker: Past, present, and future. World J Gastrointest Surg. 2020;12(12):468-490. doi: 10.4240/wjgs.v12.i12.468

[26] Yakabe T, Nakafusa Y, Sumi K, Miyoshi A, Kitajima Y, Sato S, et al. Clinical significance of CEA and CA19-9 in postoperative follow-up of colorectal cancer. Ann Surg Oncol. 2010;17(9):2349-56. doi: 10.1245/s10434-010-1004-5

[27] Bagaria B, Sood S, Sharma R, Lalwani S. Comparative study of CEA and CA19-9 in esophageal, gastric and colon cancers individually and in combination (ROC curve analysis). Cancer Biol Med. 2013;10(3):148-57. doi: 10.7497/j.issn.2095-3941.2013.03.005

[28] Zhang SY, Lin M, Zhang HB. Diagnostic value of carcinoembryonic antigen and carcinoma antigen 19-9 for colorectal carcinoma. Int J Clin Exp Pathol. 2015;8(8):9404-9.

[29] Filella X, Molina R, Grau JJ, Piqué JM, Garcia-Valdecasas JC, Astudillo E, et al. Prognostic value of CA 19.9 levels in colorectal cancer. Ann Surg. 1992;216(1):55-9. doi: 10.1097/00000658-199207000-00008

[30] Ueda T, Shimada E, Urakawa T. The clinicopathologic features of serum CA 19-9-positive colorectal cancers. Surg Today. 1994;24(6):518-25. doi: 10.1007/bf01884571

[31] Ozawa T, Ishihara S, Kawai K, Nozawa H, Yamaguchi H, Kitayama J, et al. Prognostic Significance of Preoperative Serum Carbohydrate Antigen 19-9 in Patients With Stage IV Colorectal Cancer. Clin Colorectal Cancer. 2016;15(4):e157-e163. doi: 10.1016/j.clcc.2016.04.012

[32] Shin JK, Kim HC, Lee WY, Yun SH, Cho YB, Huh JW, et al. High preoperative serum CA 19-9 levels can predict poor oncologic outcomes in colorectal cancer patients on propensity score analysis. Ann Surg Treat Res. 2019;96(3):107-115. doi: 10.4174/astr.2019.96.3.107

[33] Silveira MJ, Martins C, Cruz T, Castro F, Amorim-Costa Â, Chester K, et al. scFv biofunctionalized nanoparticles to effective and safe targeting of CEA-expressing colorectal cancer cells. J Nanobiotechnology. 2023;21(1):357. doi: 10.1186/s12951-023-02126-4

[34] Belenichev IF, Aliyeva OG, Popazova OO, Bukhtiyarova NV. Involvement of heat shock proteins HSP70 in the mechanisms of endogenous neuroprotection: the prospect of using HSP70 modulators. Front Cell Neurosci. 2023;17:1131683. doi: 10.3389/fncel.2023.1131683

[35] Calderwood SK ,Murshid A. Molecular Chaperone Accumulation in Cancer and Decrease in Alzheimer's Disease: The Potential Roles of HSF1. Front Neurosci. 2017;11:192. doi: 10.3389/fnins.2017.00192

[36] Abi Zamer B, El-Huneidi W, Eladl MA, Muhammad JS. Ins and Outs of Heat Shock Proteins in Colorectal Carcinoma: Its Role in Carcinogenesis and Therapeutic Perspectives. Cells. 2021;10(11):doi: 10.3390/cells10112862

[37] Ge H, Yan Y, Guo L, Tian F, Wu D. Prognostic role of HSPs in human gastrointestinal cancer: a systematic review and meta-analysis. Onco Targets Ther. 2018;11(351-359. doi: 10.2147/ott.S155816

[38] Gunaldi M, Kocoglu H, Okuturlar Y, Gedikbasi A, Karabulut M, Alis H, et al. Heat shock protein 70 is a useful marker for predicting colorectal cancer. J buon. 2015;20(6):1464-70.

[39] Berthenet K, Bokhari A, Lagrange A, Marcion G, Boudesco C, Causse S, et al. HSP110 promotes colorectal cancer growth through STAT3 activation. Oncogene. 2017;36(16):2328-2336. doi: 10.1038/onc.2016.403

[40] Kang M, Jeong S, An J, Park S, Nam S, Kwon KA, et al. Clinicopathologic Significance of Heat Shock Protein 60 as a Survival Predictor in Colorectal Cancer. Cancers (Basel). 2023;15(16):doi: 10.3390/cancers15164052

[41] Mori K, Toiyama Y, Otake K, Fujikawa H, Saigusa S, Hiro J, et al. Proteomics analysis of differential protein expression identifies heat shock protein 47 as a predictive marker for lymph node metastasis in patients with colorectal cancer. Int J Cancer. 2017;140(6):1425-1435. doi: 10.1002/ijc.30557

[42] Lang BJ, Guerrero-Giménez ME, Prince TL, Ackerman A, Bonorino C, Calderwood SK. Heat Shock Proteins Are Essential Components in Transformation and Tumor Progression: Cancer Cell Intrinsic Pathways and Beyond. Int J Mol Sci. 2019;20(18):4507. doi: 10.3390/ijms20184507.

[43] Hamelin C, Cornut E, Poirier F, Pons S, Beaulieu C, Charrier J, et al. Identification and verification of heat shock protein 60 as a potential serum marker for colorectal cancer. FEBS J. 2011;278(24):4845-4859. doi: 10.1111/j.1742-4658.2011.08385.x.

[44] Gunaldi M, Kocoglu H, Okuturlar Y, Gedikbasi A, Karabulut M, Alis H, et al. Heat shock protein 70 is a useful marker for predicting colorectal cancer. J BUON. 2015;20(6):1464-1470.

[45] Bakr M, Abd-Elmawla MA, Elimam H, Gamal El-Din H, Fawzy A, Abulsoud AI, et al. Telomerase RNA component lncRNA as potential diagnostic biomarker promotes CRC cellular migration and apoptosis evasion via modulation of β-catenin protein level. Noncoding RNA Res. 2023;8(3):302-314. doi: 10.1016/j.ncrna.2023.03.004

[46] Tatsumoto N, Hiyama E, Murakami Y, Imamura Y, Shay JW, Matsuura Y, et al. High telomerase activity is an independent prognostic indicator of poor outcome in colorectal cancer. Clin Cancer Res. 2000;6(7):2696-701.

[47] Garcia-Aranda C, de Juan C, Diaz-Lopez A, Sanchez-Pernaute A, Torres AJ, Diaz-Rubio E, et al. Correlations of telomere length, telomerase activity, and telomeric-repeat binding factor 1 expression in colorectal carcinoma. Cancer. 2006;106(3):541-51. doi: 10.1002/cncr.21625

[48] Terrin L, Rampazzo E, Pucciarelli S, Agostini M, Bertorelle R, Esposito G, et al. Relationship between tumor and plasma levels of hTERT mRNA in patients with colorectal cancer: implications for monitoring of neoplastic disease. Clin Cancer Res. 2008;14(22):7444-51. doi: 10.1158/1078-0432.Ccr-08-0478

[49] Lledó SM, Garcia-Granero E, Dasí F, Ripoli R, García SA, Cervantes A, et al. Real time quantification in plasma of human telomerase reverse transcriptase (hTERT) mRNA in patients with colorectal cancer. Colorectal Dis. 2004;6(4):236-42. doi: 10.1111/j.1463-1318.2004.00627.x

[50] Zhang Z, Xu L, Huang L, Li T, Wang JY, Ma C, et al. Glutathione S-Transferase Alpha 4 Promotes Proliferation and Chemoresistance in Colorectal Cancer Cells. Front Oncol. 2022;12(887127. doi: 10.3389/fonc.2022.887127

[51] Liu H, Chen X, Wang P, Chen M, Deng C, Qian X, et al. PRMT1-mediated PGK1 arginine methylation promotes colorectal cancer glycolysis and tumorigenesis. Cell Death Dis. 2024;15(2):170. doi: 10.1038/s41419-024-06544-6

[52] Brzozowa-Zasada M, Piecuch A, Bajdak-Rusinek K, Michalski M, Klymenko O, Matysiak N, et al. Glutathione Reductase Expression and Its Prognostic Significance in Colon Cancer. Int J Mol Sci. 2024;25(2):doi: 10.3390/ijms25021097

[53] Piecuch A, Kurek J, Kucharzewski M, Wyrobiec G, Jasiński D, Brzozowa-Zasada M. Catalase immunoexpression in colorectal lesions. Prz Gastroenterol. 2020;15(4):330-337. doi: 10.5114/pg.2020.101562

[54] Adachi Y, Nojima M, Mori M, Yamano HO, Sasaki Y, Nakase H, et al. Association of serum superoxide dismutase activity and the incidence of colorectal cancer in a nested case-control study. Cancer Epidemiol. 2023;87(102455. doi: 10.1016/j.canep.2023.102455

[55] Popova NV ,Jücker M. The Functional Role of Extracellular Matrix Proteins in Cancer. Cancers (Basel). 2022;14(1):doi: 10.3390/cancers14010238

[56] Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuña JM, Perez-Romero BA, Guerrero-Rodriguez JF, et al. The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases. Int J Mol Sci. 2020;21(24):doi: 10.3390/ijms21249739

[57] Gong Y, Scott E, Lu R, Xu Y, Oh WK, Yu Q. TIMP-1 promotes accumulation of cancer associated fibroblasts and cancer progression. PLoS One. 2013;8(10):e77366. doi: 10.1371/journal.pone.0077366

[58] Meng C, Yin X, Liu J, Tang K, Tang H, Liao J. TIMP-1 is a novel serum biomarker for the diagnosis of colorectal cancer: A meta-analysis. PLoS One. 2018;13(11):e0207039. doi: 10.1371/journal.pone.0207039

[59] Meng C, Yin X, Liu J, Tang K, Tang H, Liao J. TIMP-1 is a novel serum biomarker for the diagnosis of colorectal cancer: A meta-analysis. PLoS One 2018;13(11):e0207039. doi: 10.1371/journal.pone.0207039.

[60] Faivre N, Verollet C, Dumas F. The chemokine receptor CCR5: multi-faceted hook for HIV-1. Retrovirology. 2024;21(1):2. doi: 10.1186/s12977-024-00634-1

[61] Karin N. Chemokines and cancer: new immune checkpoints for cancer therapy. Curr Opin Immunol. 2018;51(140-145. doi: 10.1016/j.coi.2018.03.004

[62] Pączek S, Łukaszewicz-Zając M, Mroczko B. Chemokines-What Is Their Role in Colorectal Cancer? Cancer Control. 2020;27(1):1073274820903384. doi: 10.1177/1073274820903384

[63] Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. Nat Rev Immunol. 2017;17(9):559-572. doi: 10.1038/nri.2017.49

[64] Yue M, Chen M-M, Zhang B, Wang Y, Li P, Zhao Y. The functional roles of chemokines and chemokine receptors in colorectal cancer progression. Biomedicine & Pharmacotherapy. 2024;170(116040. doi: 10.1016/j.biopha.2023.116040

[65] Matsusue R, Kubo H, Hisamori S, Okoshi K, Takagi H, Hida K, et al. Hepatic stellate cells promote liver metastasis of colon cancer cells by the action of SDF-1/CXCR4 axis. Ann Surg Oncol. 2009;16(9):2645-53. doi: 10.1245/s10434-009-0599-x

[66] Akishima-Fukasawa Y, Nakanishi Y, Ino Y, Moriya Y, Kanai Y, Hirohashi S. Prognostic significance of CXCL12 expression in patients with colorectal carcinoma. Am J Clin Pathol. 2009;132(2):202-10; quiz 307. doi: 10.1309/ajcpk35vzjewcutl

[67] Kim J, Takeuchi H, Lam ST, Turner RR, Wang HJ, Kuo C, et al. Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival. J Clin Oncol. 2005;23(12):2744-53. doi: 10.1200/jco.2005.07.078

[68] Ottaiano A, Franco R, Aiello Talamanca A, Liguori G, Tatangelo F, Delrio P, et al. Overexpression of both CXC chemokine receptor 4 and vascular endothelial growth factor proteins predicts early distant relapse in stage II-III colorectal cancer patients. Clin Cancer Res. 2006;12(9):2795-803. doi: 10.1158/1078-0432.Ccr-05-2142

[69] Wang M, Yang X, Wei M, Wang Z. The Role of CXCL12 Axis in Lung Metastasis of Colorectal Cancer. J Cancer. 2018;9(21):3898-3903. doi: 10.7150/jca.26383

[70] Montalto FI ,De Amicis F. Cyclin D1 in Cancer: A Molecular Connection for Cell Cycle Control, Adhesion and Invasion in Tumor and Stroma. Cells. 2020;9(12):doi: 10.3390/cells9122648

[71] Binabaj MM, Bahrami A, Khazaei M, Avan A, Ferns GA, Soleimanpour S, et al. The Prognostic Value of Small Noncoding microRNA-21 Expression in the Survival of Cancer Patients: A Meta-Analysis. Crit Rev Eukaryot Gene Expr. 2020;30(3):207-221. doi: 10.1615/CritRevEukaryotGeneExpr.2020028719

[72] Jun SY, Kim J, Yoon N, Maeng LS, Byun JH. Prognostic Potential of Cyclin D1 Expression in Colorectal Cancer. J Clin Med. 2023;12(2):doi: 10.3390/jcm12020572

[73] Thoma OM, Neurath MF, Waldner MJ. Cyclin-Dependent Kinase Inhibitors and Their Therapeutic Potential in Colorectal Cancer Treatment. Front Pharmacol. 2021;12(757120. doi: 10.3389/fphar.2021.757120

[74] Ziemke EK, Dosch JS, Maust JD, Shettigar A, Sen A, Welling TH, et al. Sensitivity of KRAS-Mutant Colorectal Cancers to Combination Therapy That Cotargets MEK and CDK4/6. Clin Cancer Res. 2016;22(2):405-14. doi: 10.1158/1078-0432.Ccr-15-0829

[75] Selim NM, Mandour IA, El-sayed R, El-ghoneimy E, Farghaly MI, Abdel samie RM, et al. Association of Serum Cyclin D1 and Variations of MIR 196A2 and Deleted in Colorectal Cancer (DCC) Genes with Colorectal Cancer. Med J Cairo Univ. 2022;90(8):2767-2775. doi: 10.21608/mjcu.2022.296227

[76] Tóthová V ,Gibadulinová A. S100P, a peculiar member of S100 family of calcium-binding proteins implicated in cancer. Acta Virol. 2013;57(2):238-46. doi: 10.4149/av_2013_02_238

[77] Schmid F, Dahlmann M, Röhrich H, Kobelt D, Hoffmann J, Burock S, et al. Calcium-binding protein S100P is a new target gene of MACC1, drives colorectal cancer metastasis and serves as a prognostic biomarker. Br J Cancer. 2022;127(4):675-685. doi: 10.1038/s41416-022-01833-3

[78] Wang G, Platt-Higgins A, Carroll J, de Silva Rudland S, Winstanley J, Barraclough R, et al. Induction of metastasis by S100P in a rat mammary model and its association with poor survival of breast cancer patients. Cancer Res. 2006;66(2):1199-207. doi: 10.1158/0008-5472.Can-05-2605

[79] Liu BX, Tang CT, Dai XJ, Zeng L, Cheng F, Chen Y, et al. Prognostic Value of S100P Expression in Patients With Digestive System Cancers: A Meta-Analysis. Front Oncol. 2021;11(593728. doi: 10.3389/fonc.2021.593728

[80] Cheng YQ, Wang SB, Liu JH, Jin L, Liu Y, Li CY, et al. Modifying the tumour microenvironment and reverting tumour cells: New strategies for treating malignant tumours. Cell Prolif. 2020;53(8):e12865. doi: 10.1111/cpr.12865

[81] Geindreau M, Bruchard M, Vegran F. Role of Cytokines and Chemokines in Angiogenesis in a Tumor Context. Cancers (Basel). 2022;14(10):doi: 10.3390/cancers14102446

[82] Kaler P, Godasi BN, Augenlicht L, Klampfer L. The NF-κB/AKT-dependent Induction of Wnt Signaling in Colon Cancer Cells by Macrophages and IL-1β. Cancer Microenviron. 2009;2(1):69-80. doi: 10.1007/s12307-009-0030-y

[83] Knüpfer H ,Preiss R. Serum interleukin-6 levels in colorectal cancer patients--a summary of published results. Int J Colorectal Dis. 2010;25(2):135-40. doi: 10.1007/s00384-009-0818-8

[84] Zhao X, Lu M, Liu Z, Zhang M, Yuan H, Dan Z, et al. Comprehensive analysis of alfa defensin expression and prognosis in human colorectal cancer. Front Oncol. 2022;12(974654. doi: 10.3389/fonc.2022.974654

[85] Jeong D, Kim H, Kim D, Ban S, Oh S, Ji S, et al. Defensin alpha 6 (DEFA6) is a prognostic marker in colorectal cancer. Cancer Biomark. 2019;24(4):485-495. doi: 10.3233/cbm-182221

[86] Mothes H, Melle C, Ernst G, Kaufmann R, von Eggeling F, Settmacher U. Human Neutrophil Peptides 1-3--early markers in development of colorectal adenomas and carcinomas. Dis Markers. 2008;25(2):123-9. doi: 10.1155/2008/693937

[87] Kemik O, Kemik AS, Sumer A, Begenik H, Purisa S, Tuzun S. Human neutrophil peptides 1, 2 and 3 (HNP 1-3): elevated serum levels in colorectal cancer and novel marker of lymphatic and hepatic metastasis. Hum Exp Toxicol. 2013;32(2):167-71. doi: 10.1177/0960327111412802

[88] Bukurova Iu A, Nikitina SL, Khankin SL, Krasnov GS, Lisitsin NA, Karpov VL, et al. [Identification of protein markers for serum diagnosis of cancer based on microRNA expression profiling]. Mol Biol (Mosk). 2011;45(2):376-81.

[89] Nastase A, Pâslaru L, Niculescu AM, Ionescu M, Dumitraşcu T, Herlea V, et al. Prognostic and predictive potential molecular biomarkers in colon cancer. Chirurgia (Bucur). 2011;106(2):177-85.

[90] Nam MJ, Kee MK, Kuick R, Hanash SM. Identification of defensin alpha6 as a potential biomarker in colon adenocarcinoma. J Biol Chem. 2005;280(9):8260-5. doi: 10.1074/jbc.M410054200

[91] AB DAL, Seo MK. Has the development of cancer biomarkers to guide treatment improved health outcomes? Eur J Health Econ. 2021;22(5):789-810. doi: 10.1007/s10198-021-01290-4

[92] Loktionov A. Biomarkers for detecting colorectal cancer non-invasively: DNA, RNA or proteins? World J Gastrointest Oncol. 2020;12(2):124-148. doi: 10.4251/wjgo.v12.i2.124

[93] Passaro A, Al Bakir M, Hamilton EG, Diehn M, André F, Roy-Chowdhuri S, et al. Cancer biomarkers: Emerging trends and clinical implications for personalized treatment. Cell. 2024;187(7):1617-1635. doi: 10.1016/j.cell.2024.02.041