Azhar Int J Pharm Med Sci 2024; Vol 4 (1):1-19
Review Article

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Circulating Biomarkers of Tumor Invasiveness for Diagnosis and Prognosis of Cancer Metastasis

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Article history: Received: 01-03-2023 **Revised: 26-05-2023** **Accepted: 05-06-2023**

Abstract: Cancer is one of the serious sapping health problems that threatens humans due to its incurable nature. The only hope of beating cancer is early detection that could provide considerable management and accepted prognosis. Metastasis is the dissemination of the tumor cells from the affected organ to another which is not directly connected to the primary affected site. Once metastasis is predictable, the prognosis is poor, and mortality rate is high. Hence, early diagnosis of pre-metastatic malignant tumors could improve treatment efficacy and hence patients' survival. The past three decades had witnessed an enormous progress in the understanding of the pathophysiological basis of cancer. Disturbed metastatic biomarkers can predict the possibility of future development of metastases, disease progression and therapeutic efficacy. This review collects some of the most important biomarkers that were associated with metastasis in various types of cancer; MRP1, MMP-9, TGF-β, Vimentin, Snail-1 and TWF and E-cadherin. These biomarkers proved their ability to predict who is most at risk of developing metastasis. MRP1, MMP-9, TGF-β, Vimentin, Snail-1 and TWF has been reported to be upregulated during metastasis whereas E-cadherin has been reported to be downregulated. The close monitor of these biomarkers' levels could Provide oncologists better understanding of the disease stage and hence help them taking right decisions and begin individualized treatment strategies.

Keywords: Cancer, biomarker, metastasis, prognosis, diagnosis, E-cadherin, MRP1, MMP9, Vimentin, TGFβ, Snail-1, Twinfilin .

1. INTRODUCTION

Cancer is a major public health problem worldwide and the second leading cause of death in the world. In Egypt, age-standardized incidence rates from that registry are 96.5 and 132.6/100,000 males and 97.3 and 122.1/100,000 females. The commonest sites of cancer in males are liver (18.7%), bladder (12.7%), non-Hodgkin's lymphoma (11.0%) and trachea, bronchus, and lung (8.2%). The commonest sites in females are breast (38.8%), non-Hodgkin's lymphoma (8.5%), liver (4.6%), and ovary (4.5%)[1].

Despite the considerable advancement in new-developed therapies for cancer, the overall mortality and morbidity for cancer are high and the prognosis of patients remains disappointed. On the one hand, it might be due to that the time of diagnosing cancer is always at the advanced stage; on the other hand, clinicopathological features of cancer, such as tumor invasion, and metastasis influence the prognosis of patients with cancer. Consequently, new biomarkers that could be used effectively to anticipate the prognosis of patients with cancer are in urgent need[2].

In this research, we aim to review the current state of knowledge on metastasis and circulating biomarkers of tumor invasiveness and their potential clinical applications for the diagnosis and prognosis of cancer metastasis by summarizing the importance of some of the most recently discovered metastatic biomarkers, focusing on seven of them. This study was conducted in the period between May 2022 and March 2023. The terms "Metastatic Biomarkers, diagnosis, prognosis, and the selected individual markers as MRP1, etc." were used to locate research articles and reviews published in English over the preceding ten years. ScienceDirect and PubMed were

Cite this article: Gaballah, S., Abulsoud, A., Eldesoky, N. Circulating Biomarkers of Tumor Invasiveness for Diagnosis and Prognosis of Cancer Metastasis. Azhar International Journal of Pharmaceutical and Medical Sciences, 2024; 4(1):1-19. doi: 10.21608/AIJPMS.2023.197106.1198

DOI : 10.21608/AIJPMS.2023.197106.1198 https://aijpms.journals.ekb.eg/

among the online medical resources searched. The primary papers and reviews received the most attention.

2. METASTASIS

2.1 Definition of Metastasis

Metastasis is the growth of a secondary tumor in an area of the body that is far from the original cancer site. Tumor cells must depart their originating site, travel through the bloodstream, exert pressure on blood arteries and travel to a secondary site (Figure 2) [3].

2.2 Steps of Metastasis:

2.2.1 Dissemination of cancer cells:

As a result of continuous mistakes in chromosomes segregation during mitosis, chromosomal instability arises. Micronuclei burst due to faults in chromosomal segregation releasing genomic DNA into the cytosol, which then triggers nuclear factor kappa B (NF- κB) signaling and cytosolic DNA-sensing pathways [4].

2.2.2 Local invasion:

Cancer metastasis starts firstly by local tissue invasion around the initial tumor and degradation of the basement membrane and extracellular matrix (ECM). This step is achieved by boosting the Epithelial-mesenchymal transition (EMT) process (figure 1) [5].

The predominant mechanism affect cancer metastasis is epithelial-mesenchymal transition. Epithelial cells are immotile and strongly adhere to each other and to the ECM [6], Whereas a mesenchymal cells are more vulnerable to invasion and metastasis [7].

2.2.3 Intravasation

The infiltration of cancer cells into a blood vessel or lymphatic channel through the basement membrane (BM) is the definition of intravasation [6]. The tissues' structural limitations apply some mechanical pressure to the invasive tumor cells during intravasation. Genomic rearrangement is a result of nuclear compression and raises the risk of metastatic spread [8].

2.2.4 Circulation

Most circulating tumor cells (CTCs) circulate alone; however, some cells travel in groups with the aid of neutrophils. Additionally, Neutrophils inhibit the activation of leukocytes which improves the possibility of survival of CTC [9]. Once CTCs are in the capillaries, they undergone one of the two routes: extravasation or proliferation [10].

2.2.5 Extravasation

Circulating tumor cells get stuck when they go through tiny capillaries. This either triggers extravasation of the cell or microvascular rupture [11]. The extravasation of cancer cells is facilitated by the release of cytokines into the bloodstream and the activity of platelets [12, 13].

2.2.6 Outgrowth (colonization)

Usually a fraction of about (0.01%) of the whole cancer cells survive and develop additional tumors. Both dormancy and proliferation are options for the cells that survive. Typically, tumor cells infiltrate secondary sites and go into dormancy for a while [14].

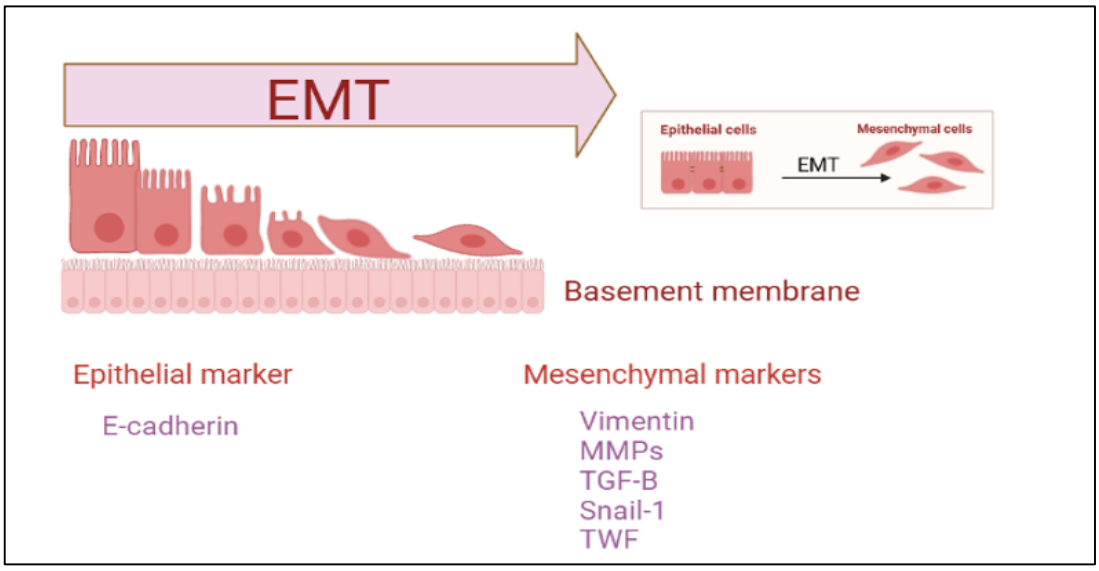

Figure 1. Epithelial-mesenchymal transition Cascade (Self constructed and designed by authors using biorender).

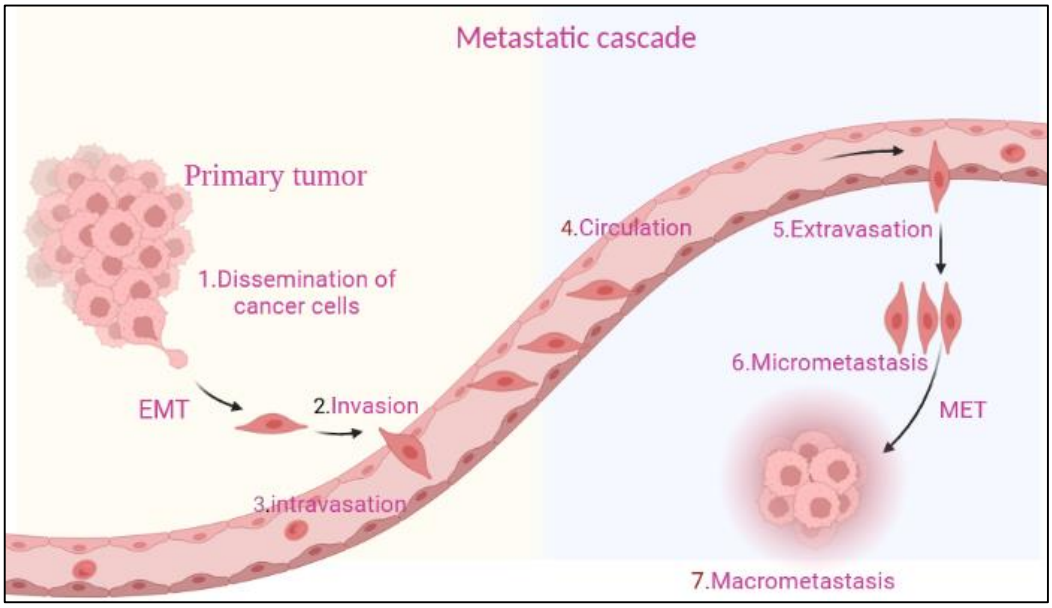

Figure 2. The metastatic cascade (Self constructed and designed by authors using biorender)

2.3 Metastatic biomarkers

The role of metastatic biomarkers whose expression levels change dynamically in tumor and have much association with tumor invasion and metastasis, has attached more attention. These molecules could serve as potential markers providing a prognostic significance for patients with cancer [15].

2.3.1 E-cadherin

E-cadherin **(E-CAD, Cadherin1, Cdh1)** is a transmembrane protein and the main building block of adherent junctions connecting epithelial tissues. It is required for integrity of tissue, junctional maintenance and dynamics [16].

Cadherin expression is regulated by progesterone and endometrial calcitonin, which has an effect on the compactness of uterine tissue and embryo implantation [17].

Adherens junctions (AJs) are signaling hubs created when the extracellular domains of cadherins connect with their cytoplasmic tails. Cadherins on nearby cells interact with one another through AJs to create a zipper like structure. E-cadherin through its cytoplasmic domain interacts with p120 catenin and beta-catenin to create complex that links AJs to the actin cytoskeleton [18]. Later, cadherins restrict cancer cell movement and progression in mature tissues by maintaining cellular polarity, tissue integrity, and homeostasis [19].

2.3.2 Motility related protein-1

Motility related protein-1 (TSPAN-29), Cluster of differentiation (CD9)) is one of the key tetraspanin family members, which orchestrate lateral interactions with other membrane proteins like integrins [20].

Motility related protein-1 has several biological activities like motility, cell migration, proliferation, fertilization, tumor metastasis and differentiation [21]. It participates in many functions, such as extracellular vesicle formation and the subsequent contribution of those structures to cell signaling [22]. Besides its physiological functions, MRP1 was found to have a major role in tumor biology field, where abnormal level causes neoplasia and metastasis [23].

2.3.3 Matrix Metalloproteinase-9 (MMP-9)

Matrix Metalloproteinase-9 (MMP-9) is a protein with endopeptidase activity. Extracellular matrix (ECM) and BM attachment are the main substrates for MMP-9. It has three repeats of fibronectin which can attach to and digest collagen which is one of the crucial elements of the basement membrane [24].

It is implicated in numerous biological processes as a result of its extracellular environment proteolytic cleavage activity. Among these biological processes are proteolytic breakdown of ECM, modification of the connections between cells and the ECM, cleavage of proteins on cell surfaces and in the extracellular environment.

2.3.4 Vimentin

Vimentin is a cytoskeletal filament protein in addition to other elements, such as microfilaments and microtubules [25]. Vimentin is widely considered as a biomarker of EMT since its level increases during tumor metastasis [26].

Vimentin has a significant role in several physiological processes and is crucial for regulating cell function. Vimentin provides anchoring effect in the organelle and keeps the normal morphology of cells. Vimentin also takes part in in cell migration, proliferation, metastasis, and invasion at the cellular level. It contributes to the growth of mammary glands, neurological system, and angiogenesis concerning function of organ. Vimentin can also regulate pathological processes like growth, wound healing and metastasis, according to many studies. In cells with high mobility, vimentin was shown to be highly expressed [27].

2.3.5 Transforming growth factor beta (TGF-β)

Transforming growth factor beta (TGF-β) is a group of released cytokines that have three different isoforms: TGFβ-1, TGFβ-2, and TGFβ-3. Each one of the TGF-β family isoforms has a unique function and expression pattern [28]. It has been hypothesized that the TGFβ-1 isoform is crucial for cancer aggressiveness [29]. It has many physiological functions including wound healing, inflammation, differentiation, and embryogenesis. Additionally, it takes part in the pathological processes like diabetes, cancer, and neurological disorders [30]. TGF-β may stimulate tumor profileration and metastasis in later stages of cancer.

2.3.6 Snail-1

Snail-1 is a zinc-finger transcription factor [31]. Snail-1 is a protein having 264 amino acids. The 2.0 kb, 3-exon SNAI1 gene, which is found on chromosome 20q13.2, encodes the Snail-1 protein in humans. Human kidney, thyroid, lungs, lymph nodes, liver, placenta and skeletal muscle tissues physiologically express Snail-1 [32, 33].

Physiologically, Snail-1 contributes to cell survival, wound healing, and embryo implantation [34]. The Snail superfamily is essential for many processes that are connected to apoptosis, metastasis, differentiation and drug resistance [35]. Snail-1 controls important genes involved in carcinogenesis [36]. It has received substantial research as a crucial EMT marker [37]. Furthermore, it is vital to fibroblast activation [36], which is important to cancer-associated fibroblasts' growth and controls tumor metastasis or immune system evasion [38].

2.3.7 Twinfilin

Twinfilin is actin binding protein. It attaches to ADP-G actin to stop G actin from binding to actin filaments [42]. TWF-1 and TWF-2 are two isoforms found in mammals that have differing subcellular locations and tissue distributions [43].

Twinfilin participates in many developmental processes, like platelet activation, cell migration, endocytosis, cell motility, and axonal growth of neurons [44]. It also controls the dynamics of cytoskeletal structure that involves interactions with actin filaments, actin monomers and is implicated in sensitivity to drugs. Furthermore, TWF is vital in regulating EMT in cancer and chemoresistance. TWF also functions as regulator of metastasis and cell cycle [45].

Table (1): The key genes involved in malignancy regulated by Snail-1

Target gene	Common function	Role of Snail-1	References
E-cadherin	Cell-cell adhesion	Gene suppression	(39)
Vimentin	Cytoskeleton building, Mesenchymal marker	Gene upregulation	(40)
MMP	ECM degradation	Gene upregulation	(41)

3. Diagnostic significance of metastatic biomarkers:

3.1 E-cadherin in cancer metastasis

Cell-cell adhesion is critically dependent on E-cadherin. Without E-cadherin, cells start to grow on top of each other initiating cancer [46]. Hence, E-cadherin might prevent epithelial cells from initially separating from the original mass of tumor. When cell junctions and cell-cell adhesion are lost, this will enable cells to move to distant places and invade adjacent tissues [47]. Additionally, the activity state of E-cadherin at the cell surface controls the cancer metastatic process (Dissemination of cancer cells, invasion, intravasation, circulation, extravasation and colonization) [48]. Therefore, cadherins are frequently inactivated or functionally inhibited

during carcinogenesis enabling the metastatic processes to occur [20].

Canel and his colleagues [49] stated that in vitro invasion and dissemination were found to increase when E-cadherin was lost demonstrating that E-cadherin inhibits cell invasion and acts as a metastasis suppressor.

Sinkevicius et al. [50] described that in lung adenocarcinoma models, loss of E-cadherin promoted tumor growth and exacerbated metastasis to lymph node or chest wall. In addition, **Siegel et al.** [51] observed in lung cancer patients with low survival rates that E-cadherin loss was associated with a high incidence of brain metastases.

Moreover, **Christou et al.** [52] found that E-cadherin loss promoted colorectal carcinoma cell (CRC) metastasis. **Drury et al.** [53] stated that tumor stage, invasion depth, and lymph node metastasis are associated with downregulated expression of E-cadherin in CRC patient tumors. Moreover, reduced E-Cadherin level has been shown in lobular breast cancer metastasis [54], diffuse gastric carcinoma [55], and pseudopapillary neoplasm of the pancreas [56].

Epithelial mesenchymal transition (EMT) has been proposed to have a strong link with aggressiveness of tumor and metastatic proclivity [57]. A meta-analysis included 1939 individuals with Head and Neck Squamous Cell Carcinoma revealed that downregulation of E-cadherin was predictive for poor survival and a considerably greater likelihood of metastatic development [58]. In another report by **Venhuizen et al.** [59] stated that loss of E-cadherin was thought to be a fundamental characteristic of invasive lobular carcinoma, with 87% of invasive lobular carcinoma and just 7% of non-lobular breast cancers were E-cadherin-negative.

3.2 Motility related protein -1 in cancer metastasis

Previous research work had reported the presence of a connection between MRP1 and cancer cell proliferation, survival, metastasis and prognosis [60]. Studies by **Baek et al.**[61] and **Liang et al.** [62] reported in acute lymphoblastic leukemia and breast cancer patients, a strong relation between increased MRP1 level and worse prognosis. Motility Related Protein-1 was proposed as a biomarker candidate for metastatic cell renal cell carcinoma (RCC), which might be used to foretell RCC's propensity for metastasis as well as to discriminate between different cancer subtypes [63]. Moreover, MRP1 was found to be overexpressed in esophageal squamous cell carcinoma tissues and its level was linked to lymph node metastasis and tumor stage [64].

Baek et al. [61] **and Huan et al.** [64] reported that in gastric and esophageal malignancies, MRP1 expression was associated with an advanced tumor stage, poor prognosis, and metastasis. They explained increased invasiveness of tumor cells resulting from the activation of signaling molecules, such as Phosphatidylinositol 4-kinase (PI4K) by the transcription of matrix metalloproteinase 2 caused by MRP1 crosslinking.

Another study by **Kim et al.** [65] stated that MRP1 was found to be positively correlated with metastasis of lymph node. Epidermal growth factor receptor (EGFR) and discoidin domain receptor (DDR1) were associated with the MRP1 which regulate cell motility. As a result of interaction with EGFR, tumor cells are able to extra- or intravasate by EGF-dependent chemotactic migration to extracellular matrix.

Baek et al. [61] stated that MRP1 overexpression accelerated bone metastasis in human breast cancer. Another two studies found a metastasis enhancing role of MRP1 in gastric carcinoma. In the first report, MRP1 expression was overexpressed in primary and metastatic gastric cancer tissues than it was in the patient's adjacent stromal non-cancerous areas. They found also a strong association of elevated MRP1 expression levels with vessel invasion [66]. In the second one, MRP1 was strongly positivity linked with lymph node metastasis and venous invasion [67].

3.3 Matrix Metalloproteinase-9 (MMP-9) in cancer metastasis

During tumor development, MMP-9 has a role in BM destruction that in turn helps in tumor invasion and metastases [68]. Matrix Metalloproteinase-9 overproduction causes the primary ECM and BM constituents to degrade, allowing tumor cells to escape promoting metastasis. Studies by **Pego et al.** [69] and **Candido et al.** [70] described MMP-9 as a tumor microenvironment motivator as it aids tumor invasion, metastasis, and angiogenesis, in addition to ECM remodeling.

Several matrix metalloproteinases have been found to be overexpressed in advanced mammary tumors, and this contributes to tumor invasion, metastasis, and a worse outcome for breast cancer [71]. In numerous studies, MMP-9 high level has been found as an indicator for invasive ductal carcinoma and has been linked to tumor level [72]. Matrix Metalloproteinase-9 was found in breast cancer cells to trigger signaling pathways that activate genes related to migration and invasion [73, 74]. In a similar study by **Li et al.** on breast cancer tissues, they found

a correlation between MMP-9 expression level, lymph node metastasis and tumor stage [75].

Furthermore, **Gobin et al.** [76] found MMP-9 significantly overexpressed in RCC metastasis and angiogenesis. **Li et al.** [77] stated that silencing of the MMP-9 expression boosted by transforming growth factor β1 (TGF-β1) blocks EMT and inhibits migration and metastasis of thyroid cancer cells as well as esophageal squamous cell carcinoma (ESCC) cells. **Jiang and Li** [78] reported that in patients with brain cancer, MMP overexpression was linked to lymph node metastasis, proposing it to be used as an indicator for brain cancer metastasis. According to reports, MMP-9 is crucial for the invasion and metastasis of gastric [79], colon [80] and lung [81] cancers.

3.4 Vimentin in cancer metastasis

Vimentin regulates cell invasion dependent on its unique structural and signaling characteristics. During cancer progression, vimentin promote metastasis via inhibition of focal adhesion-associated proteins [82]**.** Vimentin controls endothelial cells' "cuplike" shape to promote cancer metastasis. Vimentin has been shown to be necessary for in vitro cancer-associated fibroblasts' motility during collective migration [83]**.** Several investigations have also demonstrated vimentin's ability to promote EMT [27].

Numerous studies have illuminated that vimentin level was strongly related to the emergence of tumor. **Dauphin et al.** [84] stated that in 295 non-small cell lung cancer (NSCLC) patients, 145 had a vimentin-positive expression according to immunohistochemical detection results. Vimentin is primarily expressed in tissues that have undergone extensive metastatic spread. Meanwhile, the results of 193 patients' follow-up investigations revealed that expression of vimentin was strongly connected with NSCLC metastasis.

Du et al. [85] reported that vimentin overexpression indicated a lower chance of overall survival, a lower chance of being free from cancer, and more significant lymph node metastases, according to a study on 1,969 CRC patients. Furthermore, vimentin's significance in the lymphatic invasion and lymph node metastases of gastric, esophageal, and oral squamous cell carcinoma (OSCC) has been described in various instances. It has been demonstrated that OSCC lymph node metastasis was highly correlated with abnormal vimentin expression level [86]**. Bhardwaj et al.** [87] found that in OSCC originating from the sebaceous gland, elevated expression of vimentin was likewise substantially linked with metastasis of lymph node**.**

Bu and chen [88] reported that in OSCC-derived cell line Tca8113, metastasis and invasion of the OSCC were linked to the transforming growth factor 1 (TGF1)-dependent overexpression of vimentin. Blocking TGF1 reduced the capacity of these cells to migrate, pointing to vimentin's role in promoting TGF1-induced EMT.

Moreover, **Liu et al.** [89] have considered vimentin as the most optimistic marker among the five indicators concerning EMT vimentin, snail, Twist, N-cadherin and E-cadherin.

3.5 Transforming growth factor β in cancer metastasis

Many cancers have aberrant TGF-β signaling expression, including hepatocellular carcinoma, gastric, lung, and breast tumors [90]**.** Transforming growth factor promotes tumor formation by promoting EMT, invasion and metastasis [91]**.**

According to reports by **Im et al.** [92]**,** TGF-β increased gastric cancer metastasis through upregulating MMP-9**.** Furthermore, **Liu et al.** [93] stated that TGF-β was able to accelerate CRC growth and migration by inducing the EMT. Another study on CRCs by **Zhao et al.** [94] stated that greater metastasis and invasiveness were associated with decreased E-cadherin expression, and activated TGF-β Smad signalling.

Furthermore, TGF-β increased the contact between pericyte-endothelium and fibronectin, which in turn enhanced metastatic invasion of breast tumor [95]**.** Transforming growth factor β also boosted breast cancer profileration by MMP-9 dependent mechanism. Moreover, it stimulates urokinase plasminogen activator, which is important for the in-vivo spread of aggressive breast cancer [96].

Ashrafizadeh et al.[97] stated that signaling of transforming growth factor β enables bone metastasis of prostatic cancer cells, in this case it was regarded to be a diagnostic and prognostic biomarker. Its signaling contributes to metastasis of prostatic cancer cells via EMT mechanism. This was supported by another study by **Lee et al.** [98] who stated that TGF-1 exposure in human prostatic cancer cells caused EMT, enhanced invasiveness, and boosted metastasis.

Transforming growth factor beta boosts macrophages to produce vascular endothelial growth factor (VEGF), and this was thought to have promoted tumor vascularization. According to **Sun et al.,**[99] TGF activated EMT like transition in human melanoma cells via platelet-derived growth factor (PDGF) and phosphatidylinositol 3-kinase (PI3K) signalling.

In another study by **Bi et al.,** [100] TGF-/Smad signaling was enhanced, facilitating HCC progression, migration and metastasis. **Khatami** [101] stated that TGF promotes keratinocyte proliferation as well as EMT in pancreatic cells, and increases transcription of NF-κB target genes. Furthermore, **Steinbichler et al.** [102] reported that pancreatic cancer cells' exosomes motivated kupffer cells to produce TGF-β, fostering an inflammatory milieu that enhanced the pancreatic carcinoma metastasis.

Johansson et al. [103] found that breast cancer cells acquired EMT characteristics and became sensitive to the TGF-β growth stimulatory effects, promoting their metastasis to the lungs of tumor-bearing mice.

3.6 Snail- 1 in metastatic studies

Snail-1 is a vital transcription factor to EMT. It acts by suppressing E-cadherin, which causes N-cadherin protein and vimentin expression to be upregulated enabling tumor invasion [104]**.** Cancer cell metastasis could be prevented by inhibiting Snail-1 through hindering EMT and invasiveness [105]**.** Snail-1 was found in various cancer types, such as cervical, bladder, breast, hepatocellular and pancreatic carcinoma**.**

Hojo et al. [106] found that the knockdown of Snail-1 expression in an animal model suppressed the metastasis of ovarian tumors via the upregulation of MMP expression.

Kuncman et al.[107] demonstrated a substantial correlation between lymph node metastases and elevated expression levels of Snail-1, and TGF-1 in thyroid cancer patients' specimens**.** Moreover**, Wieczorek-Szukala et al.**[108] reported that there was a strong correlation between Snail-1 expression and the staging system of thyroid cancer. **Mato et al.**[109] were successful in demonstrating that patient samples with thyroid cancer in stages I and II had over 8-fold greater levels of Snail-1 expression compared to controls.

Chen et al. [110] recommended Snail-1 use in head and neck squamous cell carcinoma as a vital biomarker for lymph node metastasis. They clarified that by enhancing EMT process which favors tumor progression, in addition to the formation and collective migration of CTC clusters. They added that Snail-1 could promote angiogenesis by elevating VEGF expression [111]**.** The primary controlling factor in tumor-induced lymphangiogenesis that encourages lymph node metastasis is vascular endothelial growth factor, which is released by malignant tumor cells [112]. The VEGF boosts lymphatic vessel growth that surround the tumors to express the integrin 41, which couples with vascular cell adhesion molecule 1 to help lymph node metastasis and cancer cell migration [113].

Olmeda *et al.* [114], Mahabir *et al.* [115] **and Hemmatzadeh et al.** [116] found that Snail-1 silencing downregulated vimentin, fibronectin and MMP-9 in human breast carcinoma, malignant glioma cell lines and esophageal Cancer Cells, respectively. They stated that EMT process promotes invasiveness and migratory properties of cells by differentiation of epithelial cell to become mesenchymal cells. In this process, common epithelial indicators including E-cadherin, Mucin-1, and cytokeratin are replaced by the expression of vimentin and fibronectin, which are mesenchymal markers. They further explained that protease enzymes play a crucial part in the EMT process, which promotes tumor cells' ability to metastasize to other tissues through the extracellular matrix and its constituent parts. These enzymes participate in this process through destroying extracellular matrix constituents and dissolving the connections of connective tissues [117]**.**

Snail-1 is supposed to be necessary for lymph node metastasis in human breast cancer [118]**.** Additionally, it is believed that Snail-1 expression influences the ability of pancreatic tumor cells to metastasize [114]**.** Therefore, Snail-1 may be a suitable candidate for targeting in order to stop metastasis.

3.7 Role of twinfilin in metastasis

Many studies have been done on the role of TWF in many diseases, including malignancies. **Kaishang et al.** [119] stated that TWF could have a role in the metastasis of certain tumor types. Twinfilin has been found to promote cancer metastasis. Overexpression of Twinfilin boosts EMT process by turning on transcription factors that promote the mesenchymal lineage, like Mega karyoblastic leukemia 1(MKL1) and Serum response factor (SRF) [42]**.**

Kaishang et al. [119] stated that in adenocarcinoma of the lung and squamous cell carcinoma patients, the expression of TWF, tumour growth and lymph node metastasis are in great correlation. **Chen et al.** [120] found that increased TWF-induced aberrant EMT activation in lung adenocarcinoma causes tumor invasion, metastatic activation, the appearance of treatment resistance, and a poor prognosis. Twinfilin could alter mTOR activation to influence cell proliferation [121] where lung cancer is linked to dysregulation of serine/threonine protein kinase mTOR [122]**.**

Samaeekia et al.[42] stated that in breast cancer, the level of miR206 was downregulated, then targets

twinfillin which leads to accelerated metastasis via IL11 signaling pathway. In another two studies on breast cancer conducted by **Bockhorn et al.**[123] they found that the biomarker miR-30c for human breast tumor has a vital role in the prevention of metastasis by specifically targeting TWF, which activates EMT. In the other study, reduction of twinfilin in breast cancer cell line MDA-MB-231 cells have significantly led to less organised F-actin, decreased vinculin positive focal adhesions, and inhibited EMT and metastasis. Furthermore, **Tian et al.**[124] reported that TWF overexpression promoted breast cancer cells EMT by the increase of cyclin D1 expression in breast epithelial cells.

Table (2): Summary of the diagnostic significance of the most important studied metastatic biomarkers

Metastatic biomarker	Primary site	Secondary site	Marker level in metastasis	References
E-cadherin	▪lung adenocarcinoma	▪Lymph node or chest wall	Downregulation	**50**
	▪lung cancer	▪Brain		**51**
	▪Colorectal cancer	▪Lymph node		**52**
MRP1	▪ Breast cancer	▪Bone	Upregulation	**61**
	▪ Gastric cancer	▪ Lymph node		**66**
	▪ Esophageal squamous cell carcinoma	▪Lymph node		**64**
MMP-9	▪ Breast cancer	▪ Lymph node	Upregulation	**75**
	▪ Brain cancer	▪ Lymph node		**78**
Vimentin	▪ CRC	▪ Lymph node	Upregulation	**85**
	▪ oral, gastric, esophageal and oral squamous cell carcinoma (OSCC)	▪ Lymph node		**86**
	▪ OSCC	▪ Lymph node		**87**
TGF-β	▪Prostate cancer	▪Bone	Upregulation	**97**
	▪Breast cancer	▪Lung		**103**
Snail-1	▪Head and neck squamous cell carcinoma	▪Lymph node	Upregulation	110
	▪Breast cancer	▪ Lymph node		**118**
	▪Thyoid cancer	▪Lymph node		**107**
TWF	▪Lung cancer	▪Lymph node	Upregulation	**119**
	▪Squamous cell carcinoma	▪ Lymph node		**119**

Table (3): Prognostic significance of studied metastatic biomarkers

3.1 Clinical outcome with low E-cadherin	**Type**	**References**
Poor prognosis	Pancreatic carcinoma	125
Poor prognosis	HCC	126
Poor prognosis	OSCC	125
3.2 Clinical outcome with high MRP1	**Tumor type**	**References**
Associated with LN metastasis	Papillary thyroid carcinoma	65
Poor prognosis in particular type of the scirrhous type	Gastric cancer	67
Poor prognosis associated with LN metastasis	Cutaneous melanoma	127
Poor prognosis	Pancreatic carcinoma	128
Poor prognosis	Lobular breast carcinoma	61
Poor prognosis	Breast cancer	129
Poor prognosis associated with advanced disease	Esophageal squamous cell carcinoma	130
Good prognosis	Cervical cancer	131
Good prognosis	Gallbladder cancer	132
Good prognosis	Breast cancer	133

3.3 Clinical outcome with high MMP9 level	**Type of cancer**	**References**
Poor prognosis	Breast cancer	134
Poor prognosis	Gastric cancer	135
Poor prognosis	Colorectal cancer	136
Poor prognosis	Non small cell lung cancer	137
3.4 Clinical outcome with high vimentin level	**Type of cancer**	**References**
Poor prognosis	Endometrial cancer	139
Poor prognosis	Colorectal cancer	140
Poor prognosis	Lung adenocarcinoma	141
3.5 Clinical outcome with high TGF β level	**Type of cancer**	**References**
Poor prognosis	Prostatic cancer	97
Poor prognosis	Pancreatic ductal adenocarcinoma	138
3.6 Clinical outcome with high Snail-1 level	**Type of cancer**	**References**
Poor prognosis	Invasive ductal carcinoma	142
Poor prognosis	Hilar cholangiocarcinoma	143
3.7 Clinical outcome with high Twinfilin 1 level	**Type of cancer**	**References**
Poor prognosis	Lung adenocarcinoma	120
Poor prognosis	Lung adenocarcinoma	119

4. CONCLUSIONS

In conclusion, this research highlights the significant potential of circulating biomarkers for the diagnosis and prognosis of cancer metastasis. The development of non-invasive and cost-effective diagnostic tools that can accurately detect and monitor cancer invasiveness is urgently needed in clinical practice. Circulating biomarkers offer a promising avenue for achieving this goal, providing valuable information on disease progression, treatment response, and patient outcomes. Reduced E-cadherin expression might be a good biomarker for predicting poor prognosis, Whereas MRP1, MMP9, vimentin, TGFβ, Snail-1 and Twinfilin low level is associated with good prognosis. However, further research is needed to validate these biomarkers and to develop standardized assays for their detection and quantification. With continued advancements in this field, we hope to see the translation of circulating biomarkers into clinical practice in the near future, ultimately improving the prognosis and quality of life for cancer patients.

Funding: This article did not receive a specific grant from any funding agency in the public, commercial or not for specific sectors.

Acknowledgments: NA

Conflicts of Interest: The authors declare that they have no competing interest.

Ethical Statement: The study was performed in line with the ethical rules and policies set out by the Ethics committee of faculty of pharmacy,(Girls), Al-Azhar university (No 151).

Author Contribution: This work was carried out in collaboration between all authors**. Sara A. Gaballah:** Investigation, Formal analysis, Resources and Writing Original Draft. **Ahmed I. Eldesouky:** Validation, Software, Editing manuscript, Supervision, Management and Coordination. **Noha A. Eldesoky:** Conceptualization, Visualization, Writing Review, Editing, Supervision and gaining ethical approval.

List of Abbreviations: AJs: Adherent junctions, BM: Basement membrane, CD9: Cluster of differentiation 9, CRC: Colorectal carcinoma, CTCs: Circulating tumor cell , DDR1: Discoidin domain receptor 1, E-CAD: E- cadherin, ECM: Extracellular matrix, EGF: Epidermal growth factor, EGFR: Epidermal growth factor receptor, EMT: Epithelial mesenchymal transition, ESCC: Esophagus squamous cell carcinoma, HCC: Hepatocellular carcinoma, MKL1: Mega karyoblastic leukemia 1, MMP9: Matrix metalloproteinase 9, MRP1:Motility related protein-1, NF-κB: Nuclear factor kappa B, NSCLC: non–small cell lung cancer, OSCC: oral squamous cell carcinoma, PDGF: Platelet derived growth factor, PI3K: Phosphatidylinositol 3-kinase, PI4K: Phosphatidylinositol 4-kinase, RCC: renal cell carcinoma, SMAD: Suppressor of Mothers against Decapentaplegic, TGF-β: Transforming growth factor-beta, TGF-β1: Transforming growth factor β1,

SRF: Serum response factor, TWF: Twinfilin, VEGF: Vascular endothelial growth factor.

REFERENCES

1. Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in egypt: results of the national population-based cancer registry program. J Cancer Epidemiol. 2014; 2014: 437971. doi: 10.1155/2014/437971.

2. Lee SC, Tan HT, Chung MC. Prognostic biomarkers for prediction of recurrence of hepatocellular carcinoma: Current status and future prospects. World Journal of Gastroenterology.2014; 20: 3112–24. doi: 10.3748/wjg.v20.i12.3112.

3. Maitra A. Molecular envoys pave the way for pancreatic cancer to invade the liver.*Nature*. 2019; 567(7747): pp. 181–182.doi: 10.1038/d41586-019-00710-z.

4. Bakhoum SF, Ngo B, Laughney AM, Cavallo JA, Murphy CJ, Ly P, Shah P, Sriram RK, Watkins TBK, Taunk NK, Duran M, Pauli C, Shaw C, Chadalavada K, Rajasekhar VK, Genovese G, Venkatesan S, Birkbak NJ, McGranahan N, Lundquist M, LaPlant Q, Healey JH, Elemento O, Chung CH, Lee NY, Imielenski M, Nanjangud G, Pe'er D, Cleveland DW, Powell SN, Lammerding J, Swanton C, Cantley LC. Chromosomal instability drives metastasis through a cytosolic DNA response.Nature. 2018; 553 (7689): 467-472. doi: 10.1038/nature25432.

5. Ashrafizadeh M, Hushmandi K, Hashemi M, Akbari ME, Kubatka P, Raei M, Koklesova L, Shahinozzaman M, Mohammadinejad R, Najafi M, Sethi G, Kumar AP, Zarrabi A. Role of microRNA/Epithelial-to-Mesenchymal Transition Axis in the Metastasis of Bladder Cancer. Biomolecules. 2020; 10(8): 1159. doi: 10.3390/biom10081159.

6. Fouad YA, Aanei C. Revisiting the hallmarks of cancer. Am J Cancer Res. 2017; 7(5): 1016-1036.

7. Hu Z, Curtis C (2020): Looking backward in time to define the chronology of metastasis. Nat Commun; 11(1):1–4.

8. Denais CM, Gilbert RM, Isermann P, McGregor AL, te Lindert M, Weigelin B, Davidson PM, Friedl P, Wolf K, Lammerding J. Nuclear envelope rupture and repair during cancer cell migration.Science.2016; 352(6283): 353-8.doi: 10.1126/science.aad7297.

9. Leach J, Morton JP, Sansom OJ. Neutrophils: Homing in on the myeloid mechanisms of metastasis. Mol Immunol. 2019; 110: 69-76. doi: 10.1016/j.molimm.2017.12.013.

10. Raskov, H., Orhan, A., Salanti, A. & Gögenur, I. Premetastatic niches, exosomes and circulating tumor cells: Early mechanisms of tumor dissemination and the relation to surgery. *Int. J. Cancer*.2019; 146(12): Pages 3244-3255. doi:10.1002/ijc.32820.

11. Massagué J and Obenauf AC .Metastatic colonization by circulating tumour cells. *Nature*.2016; 529(7586): pp. 298–306.doi: 10.1038/nature17038.

12. Lambert AW, Pattabiraman DR, Weinberg RA. Emerging biological principles of metastasis. Cell .2017,168 (4):670–91. doi: 10.1016/j.cell.2016.11.037.

13. Gaertner F, Massberg S. Patrolling the vascular borders: platelets in immunity to infection and cancer. Nat Rev Immunol. 2019; 1–14. doi: 10.1038/s41577-019-0202-z.

14. Birkbak NJ, McGranahan N. Cancer genome evolutionary trajectories in metastasis. Cancer Cell. 2020; 37(1): 8–19. doi:10.1016/j.ccell.2019.12.004.

15. Chen Z, He X, Jia M, Liu Y, Qu D, Wu D, Wu P, Ni C, Zhang Z, Ye J, Xu J, Huang J. β-catenin overexpression in the nucleus predicts progress disease and unfavourable survival in colorectal cancer: a meta-analysis. PLoS One. 2013 ;8(5): e63854. doi: 10.1371/journal.pone.0063854.

16. Ceresa D, Alessandrini F, Bosio L, Marubbi D, Reverberi D, Malatesta P, Appolloni I.

Cdh4 Down-Regulation Impairs in Vivo Infiltration and Malignancy in Patients Derived Glioblastoma Cells. Int J Mol Sci. 2019; 20(16): 4028.doi: 10.3390/ijms20164028.

17. Grigorian IY, Linkova NS, Polyakova VO, Paltseva EM, Kozlov KL. Signaling molecules of the endometrium: Gerontological and general pathological aspects. Adv. Gerontol. 2016; 6: 36–43.

18. Brüser L, Bogdan S. Adherens Junctions on the Move-Membrane Trafficking of E-Cadherin. Cold Spring Harb Perspect Biol. 2017; 9(3): a029140.doi: 10.1101/cshperspect.a029140.

19. Sousa B, Pereira J, Paredes J. The Crosstalk Between Cell Adhesion and Cancer Metabolism. Int J Mol Sci. 2019; 20(8): 1933. doi: 10.3390/ijms20081933.

20. Horiguchi K, Yoshida S, Tsukada T, Nakakura T, Fujiwara K, Hasegawa R, Takigami S, Ohsako S. Expression and functions of cluster of differentiation 9 and 81 in rat mammary epithelial cells. J Reprod Dev. 2020; 66(6): 515-522. doi: 10.1262/jrd.2020-082.

20. Bruner HC, Derksen PWB. Loss of E-Cadherin-Dependent Cell-Cell Adhesion and the Development and Progression of Cancer. Cold Spring Harb Perspect Biol. 2018; 10(3): a029330. doi: 10.1101/cshperspect.a029330.

21. Yang J, Antin P, Berx G, Blanpain C, Brabletz T, Bronner M, Campbell K, Cano A, Casanova J, Christofori G, Dedhar S, Derynck R, Ford HL, Fuxe J, García de Herreros A, Goodall GJ, Hadjantonakis AK, Huang RYJ, Kalcheim C, Kalluri R, Kang Y, Khew-Goodall Y, Levine H, Liu J, Longmore GD, Mani SA, Massagué J, Mayor R, McClay D, Mostov KE, Newgreen DF, Nieto MA, Puisieux A, Runyan R, Savagner P, Stanger B, Stemmler MP, Takahashi Y, Takeichi M, Theveneau E, Thiery JP, Thompson EW, Weinberg RA, Williams ED, Xing J, Zhou BP, Sheng G, EMT International Association (TEMTIA) (2020): Guidelines and definitions for research on epithelial-mesenchymal transition. Nat Rev Mol Cell Biol; 21(6): 341-352.

22. Li C, Donninger H, Eaton J, Yaddanapudi K (2020): Regulatory Role of Immune Cell-Derived Extracellular Vesicles in Cancer: The Message Is in the Envelope. Front Immunol; 11:1525.

23. Leung KT, Zhang C, Chan KYY, Li K, Cheung JTK, Ng MHL, Zhang XB, Sit T, Lee WYW, Kang W, To KF, Yu JWS, Man TKF, Wang H, Tsang KS, Cheng FWT, Lam GKS, Chow TW, Leung AWK, Leung TF, Yuen PMP, Ng PC, Li CK (2020):CD9 blockade suppresses disease progression of high-risk pediatric B-cell precursor acute lymphoblastic leukemia and enhances chemosensitivity.Leukemia; 34(3): pp.709-720.

24. Augoff K, Hryniewicz-Jankowska A, Tabola R, Stach K. MMP9: Tough Target for Targeted Therapy for Cancer. Cancers (Basel). 2022; 14(7): 1847.doi: 10.3390/cancers14071847.

25. Usman S, Waseem NH, Nguyen TKN, Mohsin S, Jamal A, Teh MT, Waseem A. Vimentin Is at the Heart of Epithelial Mesenchymal Transition (EMT) Mediated Metastasis. Cancers (Basel).2021; 13(19): 4985. doi: 10.3390/cancers13194985.

26. Strouhalova K, Přechová M, Gandalovičová A, Brábek J, Gregor M, Rosel D. Vimentin Intermediate Filaments as Potential Target for Cancer Treatment. Cancers (Basel). 2020; 12(1): 184. doi: 10.3390/cancers12010184.

27. Paulin D, Lilienbaum A, Kardjian S, Agbulut O, Li Z. Vimentin: Regulation and pathogenesis. Biochimie. 2022; 197: 96-112. doi:10.1016/j.biochi.2022.02.003.

28. Xue VW, Chung JY, Córdoba CAG, Cheung AH, Kang W, Lam EW, Leung KT, To KF, Lan HY, Tang PM. Transforming Growth Factor-β: A Multifunctional Regulator of Cancer Immunity. Cancers (Basel).2020; 12(11): 3099. doi: 10.3390/cancers12113099.

29. Cave DD, Di Guida M, Costa V, Sevillano M, Ferrante L, Heeschen C, Corona M, Cucciardi A, Lonardo E. TGF-β1 secreted by pancreatic stellate cells promotes stemness and tumourigenicity in pancreatic cancer cells through L1CAM downregulation. Oncogene. 2020; 39(21):

4271-4285. doi: 10.1038/s41388-020-1289-1.

30. Ashrafizadeh M, Najafi M, Orouei S, Zabolian A, Saleki H, Azami N, Sharifi N, Hushmandi K, Zarrabi A, Ahn KS. Resveratrol Modulates Transforming Growth Factor-Beta (TGF-β) Signaling Pathway for Disease Therapy: A New Insight into Its Pharmacological Activities. Biomedicines. 2020 Jul 31;8(8):261. doi: 10.3390/biomedicines8080261.

31. Debnath P, Huirem RS, Dutta P, Palchaudhuri S. Epithelial-mesenchymal transition and its transcription factors. Biosci Rep. 2022; 42(1): BSR20211754. doi: 10.1042/BSR20211754.

32. Nieto MA. The snail superfamily of zinc-finger transcription factors. Nat. Rev. Mol. Cell Biol. **2002**; 3: 155–166. doi: 10.1038/nrm757.

33. Kaufhold S, Bonavida B. Central role of Snail1 in the regulation of EMT and resistance in cancer: A target for therapeutic intervention. J. Exp. Clin. Cancer Res. **2014**; 33: 62. doi: 10.1186/s13046-014-0062-0.

34. Swain SD, Grifka-Walk HN, Gripentrog J, Lehmann M, Deuling B, Jenkins B, Liss H, Blaseg N, Bimczok D, Kominsky DJ. Slug and Snail have differential effects in directing colonic epithelial wound healing and partially mediate the restitutive effects of butyrate. Am J Physiol Gastrointest Liver Physiol. 2019; 317(4): G531-G544. doi: 10.1152/ajpgi.00071.2019.

35. Wang N, Jiang R, Yang JY, Tang C, Yang L, Xu M, Jiang QF, Liu ZM. Expression of TGF-β1, SNAI1 and MMP-9 is associated with lymph node metastasis in papillary thyroid carcinoma. J Mol Histol. 2014; 45(4): 391-9. doi: 10.1007/s10735-013-9557-9.

36. Baulida J, García de Herreros A. Snail1-driven plasticity of epithelial and mesenchymal cells sustains cancer malignancy. Biochim Biophys Acta. 2015; 1856 (1): 55-61. doi: 10.1016/j.bbcan.2015.05.005.

37. Nieto MA, Huang RY, Jackson RA, Thiery JP. Emt: 2016. Cell. 2016; 166: 21 45.doi: 10.1016/j.cell.2016.06.028.

38. Kwa MQ, Herum KM, Brakebusch C. Cancer-associated fibroblasts: how do they contribute to metastasis? Clin Exp Metastasis. 2019 Apr;36(2):71-86. doi: 10.1007/s10585-019-09959-0.

39. Dong C, Wu Y, Yao J, Wang Y, Yu Y, Rychahou PG, Evers BM, Zhou BP. G9a interacts with Snail and is critical for Snail-mediated E-cadherin repression in human breast cancer. J Clin Invest. 2012; 122(4): 1469-86. doi: 10.1172/JCI57349.

40. Serrano-Gomez SJ, Maziveyi M, Alahari SK. Regulation of epithelial-mesenchymal transition through epigenetic and post-translational modifications. Mol Cancer. 2016; 15: 18.doi: 10.1186/s12943-016-0502-x.

41. Wu WS, You RI, Cheng CC, Lee MC, Lin TY, Hu CT. Snail collaborates with EGR-1 and SP-1 to directly activate transcription of MMP 9 and ZEB1. Sci Rep. 2017; 7(1): 17753. doi: 10.1038/s41598-017-18101-7.

42. Samaeekia R, Adorno-Cruz V, Bockhorn J, Chang YF, Huang S, Prat A, Ha N, Kibria G, Huo D, Zheng H, Dalton R, Wang Y, Moskalenko GY, Liu H. miR-206 Inhibits Stemness and Metastasis of Breast Cancer by Targeting MKL1/IL11 Pathway. Clin Cancer Res. 2017; 23(4): 1091-1103. doi:10.1158/1078-0432.CCR-16-0943.

43. Becker IC, Scheller I, Wackerbarth LM, Beck S, Heib T, Aurbach K, Manukjan G, Gross C, Spindler M, Nagy Z, Witke W, Lappalainen P, Bender M, Schulze H, Pleines I, Nieswandt B. Actin/microtubule crosstalk during platelet biogenesis in mice is critically regulated by Twinfilin1 and Cofilin1. Blood Adv. 2020; 4(10): 2124-2134. doi: 10.1182/bloodadvances.2019001303.

44. Stritt S, Beck S, Becker IC, Vögtle T, Hakala M, Heinze KG, Du X, Bender M, Braun A, Lappalainen P, Nieswandt B. Twinfilin 2a regulates platelet reactivity and turnover in mice. Blood. 2017; 130(15): 1746-1756. doi: 10.1182/blood-2017-02-770768.

45. Johnston AB, Hilton DM, McConnell P, Johnson B, Harris MT, Simone A, Amarasinghe GK, Cooper JA, Goode BL. A novel mode of capping protein-regulation by twinfilin. Elife. 2018; 7: e41313. doi: 10.7554/eLife.41313.

46. Mendonsa AM, Na TY, Gumbiner BM. E-cadherin in contact inhibition and cancer. Oncogene.2018; 37(35): 4769-4780.doi:10.1038/s41388-018-0304-2.

47. Cai D, Chen SC, Prasad M, He L, Wang X, Choesmel-Cadamuro V, Sawyer JK, Danuser G, Montell DJ. Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. Cell.2014; 157(5): 1146-59.doi: 10.1016/j.cell.2014.03.045.

48. Na TY, Schecterson L, Mendonsa AM, Gumbiner BM. The functional activity of E-cadherin controls tumor cell metastasis at multiple steps. Proc Natl Acad Sci U S A. 2020; 117(11): 5931-5937.doi: 10.1073/pnas.1918167117.

49. Canel M, Serrels A, Frame MC, Brunton VG. E-cadherin-integrin crosstalk in cancer invasion and metastasis. J Cell Sci. 2013; 126 (Pt 2): 393-401. doi: 10.1242/jcs.100115.

50. Sinkevicius KW, Bellaria KJ, Barrios J, Pessina P, Gupta M, Brainson CF, *et al*. E-cadherin loss accelerates tumor progression and metastasis in a mouse model of lung adenocarcinoma. *Am J Respir Cell Mol Biol*.2018; 59: 237–245.doi: 10.1165/rcmb.2017-0210OC.

51. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin* .2017; 67: 7–30. doi: 10.3322/caac.21387.

52. Christou N., Perraud A., Blondy S., Jauberteau M.-O., Battu S., Mathonnet M. E-cadherin: A potential biomarker of colorectal cancer prognosis. Oncol. Lett. 2017; 13: 4571–4576. doi:10.3892/ol.2017.6063.

53. Drury J, Rychahou PG, Kelson CO, Geisen ME, Wu Y, He D, Wang C, Lee EY, Evers BM, Zaytseva YY. Upregulation of CD36, a Fatty Acid Translocase, Promotes Colorectal Cancer Metastasis by Increasing MMP28 and Decreasing E-Cadherin Expression. Cancers (Basel). 2022; 14(1): 252. doi: 10.3390/cancers14010252.

54. Lobo J, Petronilho S, Newell AH, Coach J, Harlow G, Cruz A, Lopes P, Antunes L, Bai I, Walker E, Henrique R. E-cadherin clone 36 nuclear staining dictates adverse disease outcome in lobular breast cancer patients. Mod Pathol. 2019; 32(11):1574-1586. doi: 10.1038/s41379-019-0294-9.

55. Ansari S, Gantuya B, Tuan VP, Yamaoka Y. Diffuse gastric cancer: a summary of analogous contributing factors for its molecular pathogenicity. Int J Mol Sci. 2018; 19(8): 2424.doi:10.3390/ijms19082424.

56. Sangoi AR, Chan E, Stohr BA, Kunju LP. Invasive plasmacytoid urothelial carcinoma: A comparative study of E-cadherin and P120 catenin. Hum Pathol. 2020; 102: 54-59. doi: 10.1016/j.humpath.2020.07.006.

57. Kotiyal S, Bhattacharya S. Breast cancer stem cells, EMT and therapeutic targets. Biochem Biophys Res Commun. 2014; 453(1): 112-6.doi: 10.1016/j.bbrc.2014.09.069.

58. Yazdani J, Ghavimi MA, Jabbari Hagh E, Ahmadpour F. The Role of E-Cadherin as a Prognostic Biomarker in Head and Neck Squamous Carcinoma: A Systematic Review and Meta-Analysis. Mol Diagn Ther. 2018; 22(5): 523-535.doi: 10.1007/s40291-018-0351-y.

59. Venhuizen JH, Jacobs FJC, Span PN, Zegers MM. P120 and E-cadherin: Double-edged swords in tumor metastasis. Semin Cancer Biol.2020; 60:107-120.doi: 10.1016/j.semcancer.2019.07.020.

60. Wang Y, Liu S, Tian Y, Wang Y, Zhang Q, Zhou X, Meng X, Song N. Prognostic role of galectin-3 expression in patients with solid tumors: a meta-analysis of 36 eligible studies. Cancer Cell Int. 2018; 18(1): 1–15. https://doi.org/10.1186/s12935-018-0668-y.

61. Baek J, Jang N, Choi JE, Kim J, Bae YK. CD9 expression in tumor cells is associated with poor prognosis in patients with invasive lobular carcinoma. J Breast

Cancer. 2019; 22(1): 77–85. https://doi.org/10.4048/jbc.2019.22.e9.

62. Liang P, Miao M, Liu Z, Wang H, Jiang W, Ma S, Li C, Hu R. CD9 expression indicates a poor outcome in acute lymphoblastic leukemia. Cancer Biomark. 2018; 21(4): 781–6. https://doi.org/10.3233/CBM-170422.

63. Garner JM, Herr MJ, Hodges KB, Jennings LK. The utility of tetraspanin CD9 as a biomarker for metastatic clear cell renal cell carcinoma. *Biochem Biophys Res Commun.* 2016; 471: 21–25. doi: 10.1016/j.bbrc.2016.02.008

64. Huan J, Gao Y, Xu J, Sheng W, Zhu W, Zhang S, Cao J, Ji J, Zhang L, Tian Y. Overexpression of CD9 correlates with tumor stage and lymph node metastasis in esophageal squamous cell carcinoma. Int J Clin Exp Pathol. 2015; 8(3): 3054-61.

65. Kim T, Kim Y, Kwon HJ. Expression of CD9 and CD82 in papillary thyroid microcarcinoma and its prognostic significance. Endokrynol Pol. 2019; 70(3): 224-231. doi: 10.5603/EP.a2019.0009.

66. Hori H, Yano S, Koufuji K, Takeda J, Shirouzu K. CD9 expression in gastric cancer and its significance. J Surg Res. 2004; 117(2): 208-15. doi: 10.1016/j.jss.2004.01.014.

67. Miki Y, Yashiro M, Okuno T, Kitayama K, Masuda G, Hirakawa K,Ohira M. CD9-positive exosomes from cancer-associated fibroblasts stimulate the migration ability of scirrhous-type gastric cancer cells. BrJ Cancer. 2018; 118: 867–77.

68. Raeeszadeh-Sarmazdeh M, Do LD, Hritz BG. Metalloproteinases and Their Inhibitors: Potential for the Development of New Therapeutics. Cells. 2020; 9(5): 1313.doi:10.3390/cells9051313.

69. Pego ER, Fernández I, Núñez MJ. Molecular basis of the effect of MMP-9 on the prostate bone metastasis: A review. Urol Oncol. 2018; 36(6): 272-82.41.doi: 10.1016/j.urolonc.2018.03.009.

70. Candido S, Abrams SL, Steelman LS, Lertpiriyapong K, Fitzgerald TL, Martelli AM, Cocco L, Montalto G, Cervello M, Polesel J, Libra M, McCubrey JA. Roles of NGAL and MMP-9 in the tumor microenvironment and sensitivity to targeted therapy. Biochim Biophys Acta. 2016; 1863(3): 438-448.doi: 10.1016/j.bbamcr.2015.08.010.

71. Hajazimian S, Maleki M, Mehrabad SD, Isazadeh A. Human Wharton's jelly stem cells inhibit endometriosis through apoptosis induction. Reproduction. 2020; 159(5): 549-558.doi: 10.1530/REP-19-0597.

72. Fernández-Guinea O, Álvarez-Cofiño A, Eiró N, González LO, del Casar JM, Fernandez-Garcia B, Lamelas ML, Andicoechea A, Vizoso FJ. Low microvascular density at the tumor center is related to the expression of metalloproteases and their inhibitors and with the occurrence of distant metastasis in breast carcinomas. Int J Clin Oncol. 2013; 18(4):629-40. doi: 10.1007/s10147-012-0428-2.

73. Ran Z, Hou L, Guo H, Wang K, Li X. Expression of VEGF, COX-2 and MMP-9 in breast cancer and their relationship with ultrasound findings. Int J Clin Exp Pathol. 2018 Sep 1;11(9):4264-4269.

74. Mohammadizadeh F, Bagherian-Dehkordia M. Relationship between matrix metalloproteinase-9 and some clinicopathological prognostic factors of breast carcinoma. Am J Clin Exp Immunol. 2021; 10(1): 17-22.

75. Li H, Qiu Z, Li F, Wang C. The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. Oncol Lett. 2017; 14(5): 5865-5870. doi: 10.3892/ol.2017.6924.

76. Gobin E, Bagwell K, Wagner J, Mysona D, Sandirasegarane S, Smith N, Bai S, Sharma A, Schleifer R, She JX. A pan-cancer perspective of matrix metalloproteases (MMP) gene expression profile and their diagnostic/prognostic potential. BMC Cancer. 2019; 19(1): 581. doi: 10.1186/s12885-019-5768-0.

77. Li Y., He J., Wang F., Wang X., Yang F., Zhao C., Feng C., Li T. Role of MMP-9 in epithelial-mesenchymal transition of thyroid cancer. World J. Surg. Oncol. 2020;18: 181. doi: 10.1186/s12957-020-01958-w.

78. Jiang H, Li H. Prognostic values of tumoral MMP2 and MMP9 overexpression in breast cancer: a systematic review and meta-analysis. BMC Cancer. 2021; 21(1): 149. doi: 10.1186/s12885-021-07860-2.

79. Liu Q, Wu H, Li Y, Zhang R, Kleeff J, Zhang X, Cui M, Liu J, Li T, Gao J, Pan B, Wu W, Wang W, Zhou L, Guo J, Dai M, Zhang T, Liao Q, Lu Z, Zhao Y. Combined blockade of TGf-β1 and GM-CSF improves chemotherapeutic effects for pancreatic cancer by modulating tumor microenvironment. Cancer Immunol Immunother. 2020; 69(8): 1477-1492. doi:10.1007/s00262-020-02542-7.

80. Ruan P, Gu Y, Zhou H, Zhang W, Wang D, Liang J, Li C. Expression and clinical significance of CD74 and MMP-9 in colon adenocarcinomas. J BUON. 2020; 25(2):927-932.

81. Zhang H, Zhao B, Zhai ZG, Zheng JD, Wang YK, Zhao YY. Expression and clinical significance of MMP-9 and P53 in lung cancer. Eur Rev Med Pharmacol Sci. 2021; 25(3): 1358-1365. doi: 10.26355/eurrev_202102_24844.

82. Jiu Y, Peränen J, Schaible N, Cheng F, Eriksson JE, Krishnan R, Lappalainen P. Vimentin intermediate filaments control actin stress fiber assembly through GEF-H1 and RhoA. J Cell Sci. 2017 1; 130(5): 892-902. doi: 10.1242/jcs.196881.

83. Richardson AM, Havel LS, Koyen AE, Konen JM, Shupe J, Wiles WG 4th, Martin WD, Grossniklaus HE, Sica G, Gilbert-Ross M, Marcus AI. Vimentin Is Required for Lung Adenocarcinoma Metastasis via Heterotypic Tumor Cell-Cancer-Associated Fibroblast Interactions during Collective Invasion. Clin Cancer Res. 2018 Jan 15;24(2):420-432. doi: 10.1158/1078-0432.CCR-17-1776.

84. Dauphin M, Barbe C, Lemaire S, Nawrocki-Raby B, Lagonotte E, Delepine G, Birembaut P, Gilles C, Polette M. Vimentin expression predicts the occurrence of metastases in non small cell lung carcinomas. Lung Cancer. 2013; 81(1): 117-22. doi: 10.1016/j.lungcan.2013.03.011.

85. Du L, Li J, Lei L, He H, Chen E, Dong J, Yang J. High Vimentin Expression Predicts a Poor Prognosis and Progression in Colorectal Cancer: A Study with Meta-Analysis and TCGA Database. Biomed Res Int. 2018; 2018: 6387810.doi: 10.1155/2018/6387810.

86. Liu S, Liu L, Ye W, Ye D, Wang T, Guo W, Liao Y, Xu D, Song H, Zhang L, Zhu H, Deng J, Zhang Z. High Vimentin Expression Associated with Lymph Node Metastasis and Predicated a Poor Prognosis in Oral Squamous Cell Carcinoma. Sci Rep. 2016; 6: 38834.doi: 10.1038/srep38834.

87. Bhardwaj M, Sen S, Chosdol K, Bakhshi S, Pushker N, Sharma A, Kashyap S, Bajaj MS, Singh VK. Vimentin overexpression as a novel poor prognostic biomarker in eyelid sebaceous gland carcinoma. Br J Ophthalmol. 2020 Jun; 104(6): 879-884. doi: 10.1136/bjophthalmol-2018-313285.

88. Bu JQ, Chen F. TGF-β1 promotes cells invasion and migration by inducing epithelial mesenchymal transformation in oral squamous cell carcinoma. Eur Rev Med Pharmacol Sci. 2017; 21(9): 2137-2144.

89. Liu PF, Kang BH, Wu YM, Sun JH, Yen LM, Fu TY, Lin YC, Liou HH, Lin YS, Sie HC, Hsieh IC, Tseng YK, Shu CW, Hsieh YD, Ger LP. Vimentin is a potential prognostic factor for tongue squamous cell carcinoma among five epithelial-mesenchymal transition-related proteins. PLoS One. 2017 Jun 1; 12(6): e0178581. doi: 10.1371/journal.pone.0178581.

90. Villalba M, Evans SR, Vidal-Vanaclocha F, Calvo A. Role of TGF-β in metastatic colon cancer: it is finally time for targeted therapy. Cell Tissue Res. 2017; 370(1): 29-39. doi: 10.1007/s00441-017-2633-9.

91. Melzer C, von der Ohe J, Otterbein H, Ungefroren H, Hass R. Changes in uPA, PAI-1, and TGF-β Production during Breast Cancer Cell Interaction with Human Mesenchymal Stroma/Stem-Like Cells

(MSC). Int J Mol Sci. 2019; 20(11): 2630. doi: 10.3390/ijms20112630.

92. Im JY, Kim DM, Park H, Kang MJ, Kim DY, Chang KY, Kim BK, Won M. VGLL1 phosphorylation and activation promotes gastric cancer malignancy via TGF-β/ERK/RSK2 signaling. Biochim Biophys Acta Mol Cell Res. 2021; 1868(1): 118892. doi:10.1016/j.bbamcr.2020.118892.

93. Liu Q, Wu H, Li Y, Zhang R, Kleeff J, Zhang X, Cui M, Liu J, Li T, Gao J, Pan B, Wu W, Wang W, Zhou L, Guo J, Dai M, Zhang T, Liao Q, Lu Z, Zhao Y. Combined blockade of TGf-β1 and GM-CSF improves chemotherapeutic effects for pancreatic cancer by modulating tumor microenvironment. Cancer Immunol Immunother. 2020; 69(8): 1477-1492. doi:10.1007/s00262-020-02542-7.

94. Zhao S, Sun H, Jiang W, Mi Y, Zhang D, Wen Y, Cheng D, Tang H, Wu S, Yu Y, Liu X, Cui W, Zhang M, Sun X, Zhou Z, Peng Z, Yan D. miR-4775 promotes colorectal cancer invasion and metastasis via the Smad7/TGFβ-mediated epithelial to mesenchymal transition. Mol Cancer. 2017; 16(1): 12.doi:10.1186/s12943-017-0585-z.

95. Zonneville J, Safina A, Truskinovsky AM, Arteaga CL, Bakin AV. TGF-β signaling promotes tumor vasculature by enhancing the pericyte-endothelium association. BMC Cancer. 2018; 18(1): 670.doi:10.1186/s12885-018-4587-z.

96. Limoge M, Safina A, Beattie A, Kapus L, Truskinovsky AM, Bakin AV. Tumor-fibroblast interactions stimulate tumor vascularization by enhancing cytokine-driven production of MMP9 by tumor cells. Oncotarget. 2017; 8(22): 35592-35608. doi: 10.18632/oncotarget.16022.

97. Ashrafizadeh M, Shahinozzaman M, Orouei S, Zarrin V, Hushmandi K, Hashemi F, Kumar A, Samarghandian S, Najafi M, Zarrabi A. Crosstalk of Long Non-coding RNAs and EMT: Searching the Missing Pieces of an Incomplete Puzzle for Lung Cancer Therapy. Curr Cancer Drug Targets. 2021; 21(8): 640-665.doi: 10.2174/1568009621666210203110305.

98. Lee TK, Kim H, Song M, Lee JC, Park JH, Ahn JH, Yang GE, Kim H, Ohk TG, Shin MC, Cho JH, Won MH. Time-course pattern of neuronal loss and gliosis in gerbil hippocampi following mild, severe, or lethal transient global cerebral ischemia. Neural Regen Res. 2019; 14(8): 1394-1403.doi: 10.4103/1673-5374.253524.

98. Sun H, Miao C, Liu W, Qiao X, Yang W, Li L, Li C. TGF-β1/TβRII/Smad3 signaling pathway promotes VEGF expression in oral squamous cell carcinoma tumor-associated macrophages. Biochem Biophys Res Commun. 2018; 497(2): 583-590. doi: 10.1016/j.bbrc.2018.02.104.

99. Bi JG, Zheng JF, Li Q, Bao SY, Yu XF, Xu P, Liao CX. MicroRNA-181a-5p suppresses cell proliferation by targeting Egr1 and inhibiting Egr1/TGF-β/Smad pathway in hepatocellular carcinoma. Int J Biochem Cell Biol. 2019; 106: 107-116. doi: 10.1016/j.biocel.2018.11.011.

101. Khatami, M. Cancer Biology: Severe Cumulative Delayed Type Hypersensitivity Reactions.In: Inflammation, Aging and Cancer. Springer,Cham. 2017; pp 261-375. https://doi.org/10.1007/978-3-319-66475-0_6

102. Steinbichler TB, Dudás J, Riechelmann H, Skvortsova II. The role of exosomes in cancer metastasis.Seminars in Cancer Biology, Elsevier. 2017; pp. 170-181. doi: 10.1016/j.semcancer.2017.02.006.

103. Johansson J, Berg T, Kurzejamska E, Pang MF, Tabor V, Jansson M, Roswall P, Pietras K, Sund M, Religa P, Fuxe J. MiR-155-mediated loss of C/EBP*β* shifts the TGF-*β* response from growth inhibition to epithelial-mesenchymal transition, invasion and metastasis in breast cancer. *Oncogene*. 2013; **32**: 5614–5624. doi: 10.1038/onc.2013.322.

104. Villarejo A, Cortés-Cabrera A, Molina-Ortíz P, Portillo F, Cano A. Differential role of Snail1 and Snail2 zinc fingers in E-cadherin repression and epithelial to mesenchymal transition. J Biol Chem. 2014; 289(2):9 30-41. doi: 10.1074/jbc.M113.528026.

105. Mansoori B, Sandoghchian Shotorbani S, Baradaran B. RNA interference and its role in cancer therapy. Adv Pharm Bull. 2014; 4(4): 313–21. doi: 10.5681/apb.2014.046

106. Hojo N, Huisken AL, Wang H, Chirshev E, Kim NS, Nguyen SM, Campos H, Glackin CA, Ioffe YJ, Unternaehrer JJ. Snail knockdown reverses stemness and inhibits tumour growth in ovarian cancer. Sci Rep. 2018; 8(1):8704. doi: 10.1038/s41598-018-27021-z.

107. Kuncman W, Orzechowska M, Kuncman Ł, Kordek R, Taran K. Intertumoral Heterogeneity of Primary Breast Tumors and Synchronous Axillary Lymph Node Metastases Reflected in IHC-Assessed Expression of Routine and Nonstandard Biomarkers. Front Oncol. 2021;11: 660318. doi: 10.3389/fonc.2021.660318.

108. Wieczorek-Szukala K, Kopczynski J, Kowalska A, Lewinski A. *Snail-1* Overexpression Correlates with Metastatic Phenotype in $BRAF^{V600E}$ Positive Papillary Thyroid Carcinoma. J Clin Med. 2020; 9(9):2701. doi: 10.3390/jcm9092701.

109. Mato E, González C, Moral A, Pérez JI, Bell O, Lerma E, de Leiva A. ABCG2/BCRP gene expression is related to epithelial-mesenchymal transition inducer genes in a papillary thyroid carcinoma cell line (TPC-1). J Mol Endocrinol. 2014; 52(3): 289-300. doi: 10.1530/JME-14-0051.

110. Chen YQ, Hung CY, Wei MT, Kuo JC, Yang MH, Cheng HY, Chiou A. Snail Augments Nuclear Deformability to Promote Lymph Node Metastasis of Head and Neck Squamous Cell Carcinoma. Front Cell Dev Biol. 2022; 10: 809738.doi: 10.3389/fcell.2022.809738.

111. Chang Z, Zhang Y, Liu J, Zheng Y, Li H, Kong Y, Li P, Peng H, Shi Y, Cao B, Ran F, Chen Y, Song Y, Ye Q, Ding L. Snail promotes the generation of vascular endothelium by breast cancer cells. Cell Death Dis. 2020; 11(6): 457. doi: 10.1038/s41419-020-2651-5.

112. Chen JC, Chang YW, Hong CC, Yu YH, Su JL. The role of the VEGF-C/VEGFRs axis in tumor progression and therapy. Int J Mol Sci. 2013; 14(1): 88-107. doi: 10.3390/ijms14010088.

113. Garmy-Susini B, Avraamides CJ, Desgrosellier JS, Schmid MC, Foubert P, Ellies LG, Lowy AM, Blair SL, Vandenberg SR, Datnow B, Wang HY, Cheresh DA, Varner J. PI3Kα activates integrin α4β1 to establish a metastatic niche in lymph nodes. Proc Natl Acad Sci U S A. 2013; 110(22): 9042-7.doi: 10.1073/pnas.1219603110.

114. Olmeda D, Moreno-Bueno G, Flores JM, Fabra A, Portillo F, Cano A. SNAI1 is required for tumor growth and lymph node metastasis of human breast carcinoma MDA-MB-231 cells. Cancer Res. 2007; 67(24): 11721–31. doi: 10.1158/0008-5472.CAN-07-2318.

115. Mahabir R, Tanino M, Elmansuri A, Wang L, Kimura T, Itoh T, Ohba Y, Nishihara H, Shirato H, Tsuda M, Tanaka S. Sustained elevation of Snail promotes glial-mesenchymal transition after irradiation in malignant glioma. Neuro Oncol. 2014; 16(5): 671-85.doi: 10.1093/neuonc/not239.

116. Hemmatzadeh M, Mohammadi H, Babaie F, Yousefi M, Ebrazeh M, Mansoori B, Shanehbandi D, Baradaran B. Snail-1 Silencing by siRNA Inhibits Migration of TE-8 Esophageal Cancer Cells Through Downregulation of Metastasis-Related Genes. Adv Pharm Bull. 2018; 8(3): 437-445. doi: 10.15171/apb.2018.051.

117. Siahmansouri H, Somi MH, Babaloo Z, Baradaran B, Jadidi-Niaragh F, Atyabi F, Mohammadi H, Ahmadi M, Yousefi M. Effects of HMGA2 siRNA and doxorubicin dual delivery by chitosan nanoparticles on cytotoxicity and gene expression of HT-29 colorectal cancer cell line. J Pharm Pharmacol. 2016; 68(9): 1119-30. doi: 10.1111/jphp.12593.

118. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD, Stanger BZ. EMT and dissemination precede pancreatic tumor formation. Cell. 2012; 148(1-2): 349-61. doi: 10.1016/j.cell.2011.11.025.

119. Kaishang Z, Xue P, Shaozhong Z, Yingying F, Yan Z, Chanjun S, Zhenzhen

L, Xiangnan L. Elevated expression of Twinfilin-1 is correlated with inferior prognosis of lung adenocarcinoma. Life Sci. 2018; 215: 159-169.doi: 10.1016/j.lfs.2018.10.067.

120. Chen X, Fu Y, Xu H, Teng P, Xie Q, Zhang Y, Yan C, Xu Y, Li C, Zhou J, Ni Y, Li W. SOX5 predicts poor prognosis in lung adenocarcinoma and promotes tumor metastasis through epithelial-mesenchymal transition. Oncotarget. 2017; 9(13): 10891-10904.doi: 10.18632/oncotarget.22443.

121. Li L, Liu L, Qu B, Li X, Gao X, Zhang M. Twinfilin 1 enhances milk bio-synthesis and proliferation of bovine mammary epithelial cells via the mTOR signaling pathway. Biochem Biophys Res Commun. 2017; 492(3): 289-294.doi: 10.1016/j.bbrc.2017.08.130.

122. Wang Y, Liu S, Tian Y, Wang Y, Zhang Q, Zhou X, Meng X, Song N. Prognostic role of galectin-3 expression in patients with solid tumors: a meta-analysis of 36 eligible studies. Cancer Cell Int. 2018; 18(1): 1–15. https://doi.org/10.1186/s12935-018-0668-y.

123. Bockhorn J, Dalton R, Nwachukwu C, Huang S, Prat A, Yee K, Chang YF, Huo D, Wen Y, Swanson KE, Qiu T, Lu J, Park SY, Dolan ME, Perou CM, Olopade OI, Clarke MF, Greene GL, Liu H. MicroRNA-30c inhibits human breast tumour chemotherapy resistance by regulating TWF1 and IL-11. Nat Commun. 2013; 4: 1393.doi: 10.1038/ncomms2393.

124. Tian C, Zeng S, Luo J. MCTS1 Directly Binds to TWF1 and Synergistically Modulate Cyclin D1 and C-Myc Translation in Luminal A/B Breast Cancer Cells. Onco Targets Ther. 2020; 13: 5353-5361. doi: 10.2147/OTT.S255675.

125. Wang P, Zhu Z. Prognostic and Clinicopathological Significance of E-Cadherin in Pancreatic Cancer Patients: A Meta-Analysis. Front Oncol. 2021; 11: 627116. doi: 10.3389/fonc.2021.627116.

126. Chen J, Zhao J, Ma R, Lin H, Liang X, Cai X. Prognostic significance of E-cadherin expression in hepatocellular carcinoma: a meta-analysis. PLoS One. 2014; 9(8): e103952. doi: 10.1371/journal.pone.0103952

127. Lucarini G, Molinelli E, Licini C, Rizzetto G, Radi G, Goteri G, et al. Tetraspanin CD9 expression predicts sentinel node status in patients with cutaneous melanoma. *Int J Mol Sci*. 2022; 23: 4775. doi: 10.3390/ijms23094775.

128. Han X, Zhang WH, Gao HL, Li TJ, Xu HX, Li H, Li PC, Wang X, Yu XJ, Wang WQ, Liu L. Neoadjuvant chemotherapy endows CD9 with prognostic value that differs between tumor and stromal areas in patients with pancreatic cancer. J Clin Lab Anal. 2022; 36(7): e24517. doi: 10.1002/jcla.24517.

129. Kwon HJ, Choi JE, Kang SH, Son Y, Bae YK. Prognostic significance of CD9 expression differs between tumour cells and stromal immune cells, and depends on the molecular subtype of the invasive breast carcinoma. Histopathology. 2017; 70:1155–65. doi: 10.1111/his.13184.

130. Huan J, Gao Y, Xu J, Sheng W, Zhu W, Zhang S, Cao J, Ji J, Zhang L, Tian Y. Overexpression of CD9 correlates with tumor stage and lymph node metastasis in esophageal squamous cell carcinoma. Int J Clin Exp Pathol. 2015; 8(3): 3054-61. doi: 10.1034/j.1600-0714.2001.

131. Sauer G, Windisch J, Kurzeder C, Heilmann V, Kreienberg R, Deissler H. Progression of cervical carcinomas is associated with down-regulation of CD9 but strong local re-expression at sites of transendothelial invasion. Clin Cancer Res. 2003; 9: 6426–31.

132. Zou Q, Xiong L, Yang Z, Lv F, Yang L, Miao X. Expression levels of HMGA2 and CD9 and its clinicopathological significances in the benign and malignant lesions of the gallbladder. World J Surg Oncol. 2012; 10: 92. doi: 10.1186/1477-7819-10-92.

133. Miyake M, Nakano K, Ieki Y, Adachi M, Huang CL, Itoi S, Koh T, Taki T. Motility related protein 1 (MRP-1/CD9) expression: inverse correlation with metastases in

breast cancer. Cancer Res. 1995; 55(18): 4127-31.

134. Zhang QW, Liu L, Chen R, et al. Matrix metalloproteinase-9 as a prognostic factor in gastric cancer: a meta-analysis. Asian Pac J Cancer. 2012; 13: 2903–8.

135. Li CY, Yuan P, Lin SS, et al. Matrix metalloproteinase 9 expression and prognosis in colorectal cancer: a meta-analysis. Tumour Biol. 2013; 735–41.

136. Peng WJ, Zhang JQ, Wang BX, et al. Prognostic value of matrix metalloproteinase 9 expression in patients with non-small cell lung cancer. Clin Chim Acta. 2012; 413:1121–6.

137. Zhao J, Liang Y, Yin Q, Liu S, Wang Q, Tang Y, Cao C. Clinical and prognostic significance of serum transforming growth factor-beta1 levels in patients with pancreatic ductal adenocarcinoma. Braz J Med Biol Res. 2016; 49(8): e5485. doi: 10.1590/1414-431X20165485.