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ANALYSIS OF REGULATORY MOLECULES THAT MEDIATE METASTASIS AND ANGIOGENESIS IN HUMAN CANCER

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ABSTRACT

Aberrant expression of intracellular regulatory molecules promotes the progression and development of many human carcinomas. This review article highlights recent experimentally-derived observations that demonstrate the significance of critical regulatory molecules such as microRNAs in different types of cancer. Targeting molecules that lead to negative pathophysiological phenotypes, such as angiogenesis and metastasis may proffer effective strategies to attenuate cancer progression and associated cancer-related clinical outcomes in humans.

Keywords: Cancer, Metastasis, Angiogenesis, Antitumor

Abbreviations: LNCaP: Lymph node carcinoma of the prostate; CRAd: Conditionally replicative adenovirus; DYRK2: Dual-specificity tyrosine phosphorylation-regulated kinase 2; CAM:Cell adhesion molecule; TGF-β: Transforming growth factor beta; PSA: Prostate-specific antigen;PICK1: Protein interacting with C-Kinase 1; MiRNA: MicroRNA; SOX4: Sex-determining regionY-related(SRY) high-mobilitygroup box 4; Ang1: Angiopoietin-1; VEGFR:Vascular endothelial growth factor receptor; FGFR1:Fibroblast growth factor receptor 1;HCC: Hepatocellular carcinoma; MAPK/ERK: Mitogen-activated protein kinase/extracellular signal-regulated kinase; PI3K: Phosphatidylinositol3-kinase; HOXD3: HomeoboxD3; CRISPR: Clustered regularly interspaced short palindromic repeats.

INTRODUCTION

Recent advances in human cancer etiology have given rise to successful antitumor therapeutic strategies. Selective therapies are made possible from experimental investigations that explore underlying oncogenic processes that mediate transformative events in different cell types. Previous reviews have focused on the Janus kinase-Signal transducer and activator of transcription

(JAK-STAT) pathway and suppressors of cytokine signaling (SOCS) proteins (1,2). This review seeks to analyze other important regulatory molecules that contribute to cancer progression in humans. Studies examining altered gene and protein expression are instrumental in elucidating theirpotential significance in tumor-promoting mechanisms. Research into the mechanisms regulating the production and function of regulatory molecules that promote cancer may also lead to the development of more accurate diagnostic tests. Specifically, in-depth studies that can correlate oncogenic molecule expression with distinct stages of a particular type of cancer will open the door to stage-specific drugs that may enhance treatment methods and improve prognostic outcomes.

Metastasis

Metastasis occurs when cancer cells traverse to secondary sites in the body and affect distant body systems that are different from the original carcinogenic tissue. Metastatic tumors continue to grow in ancillary regions and contribute to high morbidity and mortality rates. In terms of nomenclature, metastatic tumors retain the name of their primary site of origin (3,4). A wide array of signal transduction pathways and regulatory molecules mediate the chemotactic migration and colonization of tumor cells in different body regions.Responding to metastatic cancer is problematic because the original location of the tumor, final destination, cell type, and size of the initial and distal tumor all play a role in the selection of treatment options. The conversion of epithelial cells to mesenchymal cells and the loss of cell adhesion facilitate cancer cell invasion and therefore enhances metastatic potential. The epithelial-mesenchymal transition (EMT) isthe main process by which epithelial cells are transformed from nonmotile, closely packed cellular tissue to loosely-associated, irregularly shaped cells that have developed stem cell features (5). The morphological and cellular modificationssuch as differential expression mechanisms of cell-surface proteins and other molecules that mediate intercellular connections, allow metastatic cells to avoid detection of the human immune system as they travel throughout the

body. The decrease in immune detection and response enhances the survivability of tumor cells and leads to an increase in cancer-related deaths.

Several molecules such as SNAIL, GATA1, and DYRK2 have been implicated in the EMT observed in different types of human cancers (6-8).In breast cancer, DYRK2 has been shown to mediate SNAIL degradation via serine phosphorylation. Adenocarcinoma immune-histochemical data demonstrated that DYRK2 expression was inversely proportional to SNAIL expression, suggesting that DYRK2 localization negatively alters SNAIL expression. SNAIL is a transcriptional repressor that plays a role in blocking e-cadherin expression, epithelial proteins, and stimulating expression of mesenchymal surface proteins on cancer cells. Decrease in SNAIL functionality disrupts the epithelial-mesenchymal transition in breast cancer and ovarian serous adenocarcinoma. An increase in DYRK2 expression leads to clinicopathologically favorable outcomes in cancer patients (8, 9).

A fairly recent study involving a GATA1 mutant construct provided evidence that the transcription factor plays a role in mediating the epithelial-mesenchymal transitionin breast cancer (6). Chromatin immunoprecipitation (ChIP) assay data in breast cancer cell lines demonstrated that GATA1 binds to the e-cadherin promoter and attenuates transcriptional activity. Moreover, Li et al. (6) demonstrated that e-cadherin repression involves phosphorylation of GATA1 serine residues by p21-activated kinase 5 (PAK5) and that mutant GATA1 expression in MCF-7 cells was necessary to reverse-e-cadherin promoter activity to physiologic levels. Xie et al. employed transwell assays and showed that MDA-MB-231 cells overexpressing GATA1 could efficiently migrate through the transwell and that high-GATA1 expressing cells migrated more effectively than low-GATA1 expressing cells.GATA1 has also been shown to increase cell proliferation.MCF-7 cells overexpressing GATA1 exhibited higher growth when compared to shGATA1-containing MCF-7 cells and negative control cells (7). Investigatory evidence delineated in the above studies linking GATA1 to multiple events that convert breast cancer cells to uncontrolled migrating tumor cells point to GATA1 as an excellent molecular candidate to target in the fight against breast cancer.

The evidence that multiple proteins target e-cadherin expression reaffirms the importance of the cell adhesion molecule (CAM) as a significant metastatic factor and potentially paramount diagnostic and prognostic biomarker in several types of human cancer. Surprisingly,

in breast cancer, e-cadherin expression is often more potent in tumor cells at secondary distal sites compared to the primary tumor. This data suggests that e-cadherin may perform different pathological functions as cells migrate away from the initial carcinogenic site. Given different expression levels of e-cadherin at different locations, it may be possible to differentiate clinical stages in various types of cancers simply by the identification and quantification of e-cadherin levels in tumors. Moreover, immunohistochemical appraisal demonstrates that different e-cadherin expression patterns exist for ductal carcinomas and lobular carcinomas (10).

Metastatic suppressors are designed to disrupt the biochemical, anatomical, physiological, and mechanical factors that induce tumor cell migration. Metastatic suppressor genes such as KISS1, GAS1, and BRMS1 code for advantageous proteins that interfere with cellular programs associated with cell motility and proliferation (11-13). Kisspeptin, the protein product of the metastatic suppressor gene KISS1, is important in breast cancers and has been shown to restrict breast cancer brain metastases and renders breast cancer cells more susceptible to conditionally replicative adenoviral (CRAd) therapy under experimental conditions (11). The transforming growth factor beta (TGF-β) signaling pathway is also implicated in human cancer metastasis and data suggests that TGF-β circuitry may be a promising target to suppress migration of tumor cells (14). PICK1 (protein interacting with PRKCA 1), a negative regulator of TGF-β, has been shown to control the ability of prostate cancer to metastasize to bone tissue. Initially, it was established using immunohistochemistry that PICK1 expression was reduced in prostate cancer tissues isolated from prostate cancer patients. Further, reduced PICK1 expression in patients correlated positively with prostate-specific antigen (PSA) levels from cancer-stricken patients. Investigators also demonstrated that when PICK1 was overexpressed in PC-3 cells that metastasis was suppressed. PICK1 downregulation in prostate tissues is believed to be regulated by the microRNA, miR-210-3p (15).

MicroRNAs (miRNA or miR) are non-coding oligoribonucleotides found in eukaryotes and viruses that facilitate gene silencing mechanisms. MicroRNA molecules have been discussed as potential biomarkers and targets of disease for several decades and continue to be of great enthusiasm to clinical oncologists (16).Numerous studies have shown that miRNAs both strengthen and weaken metastatic intracellular signals in many human carcinomas. A more detailed understanding of the biological mechanisms involved in miRNA

interaction will aid in the comprehension of the overall cancer development and progression process. MicroRNA-132/212 upregulation in prostate cancer cells and tissues was shown to block the epithelial-mesenchymal transition in these cells and shown to attenuate LNCaP migration in transwell assays. Comparative real-time quantitative polymerase chain reaction (RT-qPCR) analysis of prostate cancer tissues and benign prostatic hyperplasia tissues confirmed that both miR-132 and miR-212 were suppressed in human prostate cancer tissues compared to the benign tissue (17).Specifically, overexpression of miR-132/212 negatively affected TGF-β induced EMT in prostate cancer cells. Luciferase reporter assay data identified the transcription factor SOX4, whose expression is stimulated by TGF-β,as the main target of microRNA-132/212 (18).These data offer hope into the potential therapeutic application of miRNA technology to abolish the metastatic progression of human cancer cells.

Angiogenesis

Angiogenesis is a complex process of blood vessel formation from preexisting blood vessels and is a defining event in metastasis and other diseases (19). The ability to acquire oxygen and other nutrients is an essential function of blood vessels and is indispensable for normal biological function as well as tumor growth and survival. Angiogenesis is an important physiological process for the sustainability of tumor cells that occurs in a multistage process (e.g., vessel branching, maturation, and latency) (19). Controlling angiogenesis in human cancer cells represents a prospective strategy to inhibit the growth and spread of cancer tissue.

Major angiogenic factors include vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), platelet-derived growth factor (PDGF), and angiopoietin 1 (20,21).These molecules represent major angiogenic targets for which drugs are currently being designed and tested. Michael et al. showed that angiopoietin-1 abrogation using a transgenic mouse model resulted in an increase in breast tumor metastasis to lung tissue. Angiopoietin-1 is believed to stimulate cell-surface receptors on endothelial cells and promote a sturdier vasculature system. The loss of angiopoietin-1 enhances a cells susceptibility to metastatic changes (20). A comparative immune-histochemical study examining the expression level of angiopoietin-1 in metastatic breast cancer patients and healthy human subjects will provide a clearer view of the role of angiopoietin 1 in mediating metastatic progression. Functional studies involving angiopoietin 1 siRNA may also provide additional evidence regarding the role of

angiopoietin 1 to modify metastasis in human cell lines. Recently, researchers examined the efficacy of using anlotinib to treat metastatic cancer. Anlotinib is a receptor tyrosine kinase inhibitor that targets multiple pro-angiogenic factors including vascular endothelial growth factor receptor type (VEGFR) 2/3 and fibroblast growth factor receptor 1(22). Anlotinib disrupted the activation of VEGFR2, PDGFRβ, and FGFR1 and thereby produced anti-angiogenic effects such as cell migration reduction and suppression of capillary-like tubes in endothelial cells. Data also supports the conclusion that anlotinib is a more potent inhibitor of angiogenesis than sunitinib, sorafenib, and nintedanib (22).

MicroRNAs have also shown promise as potential anti-angiogenic targets in recent investigations (23). Wang et al. (24) presents a review of putative therapeutic miRNAs that have anti-angiogenic properties and show that miRNAs regulate tumorigenic activity of endothelial cells via cell-autonomous and non-cell-autonomous mechanisms. Chen et al. (25) reported that in endometrial carcinoma microRNA-29b reduces angiogenesis primarily by targeting vascular endothelial growth factor A (VEGFA) through the signaling pathways, MAPK/ERK and PI3K/Akt.HOXD3 has recently been shown to be a target of miR-203a in human hepatocellular carcinoma cells (26). HOXD3 is a highly conserved transcription factor that mediates morphogenesis and cell adhesion and is found in multicellular organisms, including humans.HOXD3 enhances VEGFR expression by binding to the VEGFR promoter region resulting in angiogenesis and cancer progression. Specifically, miR-203a inhibits both metastasis and angiogenesis by negatively regulating HOXD3 expression in HCC cells. Overexpression studies of HOXD3 in SMMC-7721 and Hep3B cells lead to invasive, metastatic, and angiogenic phenotypes, while knockdown of HOXD3 lead to suppression of the malignant physiological changes in HCCs. Overexpression of miRNA-203a in cells containing normal levels of HOXD3 was sufficient to produce results observed in HOXD3 knockdown experiments (26). Additionally, promoters of metastasis such as MMP9 and N-cadherin were upregulated as confirmed by western blotting analysis following an increase in HOXD3 expression. The data in the previously described study portend a model of miRNA inhibition that involves HOXD3 targeting with concomitant modification of angiogenic and metastatic factors.In addition to hepatocellular carincoma, HOXD3 has also appeared in literature associated with breast cancer, ovarian cancer, and prostate cancer (27-29). The effect of miR-

203a mimics and overexpression studies in early-late stage cancer patients will offer additional proof regarding the potential therapeutic value of employing miR-203a to treat human cancers.

A previous report examining hepatocellular carcinoma also demonstrated that microRNA-26a inhibits angiogenic processes in cancer cells and animal models by disrupting a phosphoinositide 3-kinase pathway (30). Expression of miR-26a in nude mice obstructed tumor growth and vasculogenesis.MicroRNA-20a was shown to promote angiogenesis and VEGFA expression in the breast cancer cell lines, MCF7 and MDA-MB-231. Treatment with anti-miR-20a ameliorated mean mesh size in MDA-MB-231 cells following tube-formation in vitro assays, suggesting that miR-20a plays a role in promoting angiogenesis and that miR-20a may offer clinical prognostic value when evaluating invasive breast cancer (31).Additionally, investigations of microRNA-155-5p in HCC have led researchers to believe that miRNA-155-5p is a pro-angiogenic factor. MicroRNA-155-5p is significantly overexpressed in HCC rat models and HCC cell lines (32). In patients with malignant colorectal cancer, circulating plasma levels of miRNA-155-5p is associated with a decrease in patient survival (33). Recently, researchers examined the role of microRNA-32-5p in multidrug resistance in HCC. Using Bel/5-FU (multidrug-resistant cell line) and Bel7402 (sensitive cell line) it was determined that exosomal miRNA-32-5p translocation from resistant cells to sensitive cells mediates the ability of cells to withstand the deleterious effects of HCC drug treatment (34). We have shown that miRNA molecules can both enhance and abrograte angiogenesis. We have also provided experimental evidence that miRNAs offer promising candidates as biomarkers and therapeutic targets.

CONCLUSIONS

The physiological distribution and temporal expression of signaling and regulatory molecules is necessary to control cell growth, development, differentiation, homeostasis, and metabolism.

Aberrant expression of critical biological molecules promotes disease in living organisms.

Further, life-threatening consequences develop when signaling circuits fail to coordinate specific biological mechanisms, and when essential molecules are expressed in supra physiologic concentrations in various tissues. Recently obtained experimental data supports the idea that direct and indirect crosstalk mechanisms among multiple intracellular signaling pathways are involved in cancer development and progression. Molecular

crosstalk processes in human tumors is not surprising given the complexity of the biological events necessary to sustain unregulated cell growth.

This type of molecular communication among cells, tissues, and organs adds to the difficulty in designing drugs to target specific processes. Targeting one molecule or one pathway may be an insufficient scheme to treat various cancers. An understanding of the integration of different signaling pathways and regulatory molecules using knockdown studies and functional assays may create a clearer picture regarding molecular elements that produce carcinogenic characteristics in human cells and tissue.

New and exciting molecular technologies such as the clustered regularly interspaced short palindromic repeats (CRISPR) genome editing technique recently demonstrated the efficacy of targeting an important signaling molecule to ameliorate cell growth. Researchers utilized the CRISPR/Cas9 system to target the androgen receptor signaling pathway and androgen receptor gene in the LNCaP prostate cancer cell line. Following androgen receptor disruption, the androgen-sensitive human prostate cancer cells displayed a statistically significant reduction in cell growth compared to control cells (35).The decrease in cell proliferation was shown to be caused by cellular apoptosis. While positive clinicopathologic findings from in vitro experiments are encouraging, efficacy data employing cutting-edge treatment strategies from cancer patients is needed and far more compelling. While the literature is growing regarding the principal oncogenic molecules that lead to poor survival outcomes, the tenebrous complexity of the interacting molecular factors, signaling pathways, and crosstalk mechanisms that drive angiogenesis and metastasis necessitate the need for the synthesis of detailed signaling molecular maps using meta-analytical procedures that chart the functional role of critical proteins and genes that have been shown to impact deleterious symptoms and mortality rates for specific cancers. Comprehensive maps will benefit researchers and physicians and potentially expedite valuable chemotherapeutic strategies.

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