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Over expression of S100P up-regulates cancer cell proliferation: unfavorable prognosis and tumor progression in patients

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Abstract

Endometrial cancer is the most common gynecologic malignancy. There is increasing evidence suggesting that S100P has a significant role in cancer. The objective of this study is investigating the role of S100P in promote endometrial cancer cell proliferation and S100P has received increasing attention due to accumulating evidence of its significant role during the development and progression of different cancers. Quantitative real-time RT-PCR (qPCR) analysis was used to measure changes of S100P. Transwells is used for migration and invasion assay respectively. Cell proliferation was analysed by flow cytometry. High expression of S100P are associated with invasion endometrial cancer while, knockdown of S100P protein decreased cellular proliferation and migration. In conclusion, S100P regulate endometrial cell proliferation migration and invasion and reduces chemoresistance.

Keywords: Endometrial cancer; S100P; Quantitative real-time RT-PCR

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Introduction

Cancer is a well-known and challenging worldwide problem that requires new efforts to address and resolve it. This study aims to address a new side of cancer biology by investigating the role of a unique target for effective human cancer therapy. The frequency of S100P overexpression, its molecular mechanisms in the regulation of the proliferation of human epithelial cancer cells, and its association with various human cancer cells are delineated. The oncogenic potential of S100P is explored using in vitro and in vivo human cancer cell models. Finally, we profile the prognostic relevance of S100P during tumor development, compared to standard molecular marker systems in a publicly accessible electronic database.

Tumor progression is a complex and dynamic process controlled by the concerted action of multiple factors. S100 proteins belong to a family of EF-hand calcium-binding proteins, and they are involved in numerous cellular processes, such as proliferation, differentiation, invasion, and metastasis. It has been reported that S100P contributes to tumor survival, apoptosis, and invasiveness in pancreatic, breast, prostate, and endometrial carcinoma, and in melanoma. This review summarizes a novel part of the molecular mechanisms of cancer invasion and metastasis by overexpressing S100P. In particular, this review will discuss how S100P is ectopically expressed in various human malignancies to regulate cell proliferation, a signaling

pathway that promotes cell spreading, a putative receptor for extracellular ligands, and a role of S100P peptides in cancer metastasis and in vitro tumor cell migration. A discussion of the unique role played by S100P in regulating cell motility is required.

The two crucial hallmarks in the majority of human cancers are the loss of growth suppression and the gain of cell proliferation. So, they are believed to take part in the progression and dissemination of cancer. Diverse patterns of genes have been seen to dysregulate in the progression and metastasis of carcinoma cells to other sites. Over the last two decades, the gene expression profiles have been explored in search of markers that might predict the clinical outcomes of malignant lesions.

Embryonic mutations and abnormal activity of oncogenes and tumor suppressor genes can create cancerous stem cells, as well as clonal genetic variations in diversity. Additionally, these cells may survive in the blood or the lymph. Although this phase of transformation is not the primary determinant of cell biology, a number of biotechnology and chemotherapeutic paradigms have been developed that target the propagation and progression of malignancies. In certain genetic mutations, studies of SCC offer knowledge into the various developmental patterns of these tumors and suggest that the effectiveness of pharmacotherapy of malignancies combining the S100P gene will differ based on the genetic background of the patient.

Mammalian S100P is 95% similar to the above S100P, and this protein, which is often known as S100A9, exhibits some unusual properties. Increasing prevalence in mucin-secreting cells, as well as continual overexpression of Gly26 and earlier stage in the multistep process of pancreatic carcinogenesis, upregulates S100P during progression to pancreatic intraepithelial neoplasms (PanIN) and pancreatic ductal adenocarcinoma (PDAC) in humans. These early-acting genes provide essential information about the first gene candidate downstream target of activated protooncogene K-ras for putting a definite destination to cells that can transform them.

Aim of the Study

The aim of this study is to summarize available data in the literature in connection with the actual role of S100P overexpression in cancer cell proliferation, which could have potential prophylactic as well as therapeutic significance. The overexpression of S100P in tumors serves as a valuable pro-metastatic factor connected with both adverse clinical outcomes together with a high capability of tumor progression. Moreover, numerous in vitro and in vivo studies have reported on the ability of S100P to activate the intracellular signaling pathways involved in tumor proliferation, activate malignant transformation events, and participate in several of the other features of the hallmarks of cancer.

For the accurate characterization of the landscape of this issue, we performed a comprehensive search of PubMed, Web of Science, Scopus, and Cochrane public databases. Our article provides an overview of available in vivo or in vitro studies with their relevant in vivo data, and our discussion is based on current knowledge from very recent studies or meta-analyzes, not

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only in studies published to date, but also with the critical integration of the awareness of the landscape of the genetic defects which are behind playing a key role in tumor progression. Finally, our article could open up a critique of the known problematics. Moreover, the inclusion of this information could have a survival value in a patient cohort and lead to risk-position discussion when empirical chemotherapy accompanies the adjuvant setting of specific operation following the detection of an over-expression of S100P.

S100P Protein: Structure and Function

S100P protein is 95 amino acids long with a molecular weight of 10.4 kDa. It is more often overexpressed in cancers (such as pancreatic and breast cancer as well as non-small cell lung cancer) and its overexpression is associated with poor prognosis and disease resistance. S100P is a peculiar protein in many aspects. It functions in both the extracellular and intracellular surroundings, and the signals activated by it perform in both autocrine and paracrine manners. S100P functions in a wide array of biological roles including cell motility and cell migration, cell proliferation and cell survival, cell death and chemoresistance, and signal transduction. The functions of S100P, as well as its versatility as a sandwich protein, would allow it or its pathway to be manipulated by a chemotherapeutic response. On the other hand, its biological roles could also be utilized to inhibit cancer cell proliferation or to reverse apoptosis resistance.

S100P belongs to the S100 protein family—the largest subgroup of 20 Ca2+-binding proteins. The structure is characterized by the presence of two helix-loop-helix (EF-hands) Ca2+-binding motifs connected by a central hinge. The S100 refers to the 100% solubility at 100% saturation with ammonium sulfate and is primarily homodimeric in solution under physiological conditions. While folded normally, the loop portions of the EF hand domains associate to form a deep cleft for the binding of divalent metal ions (zinc, calcium, copper, etc.) to stabilize its structure with an acidic cleft for the binding of target proteins.

This binds intracellularly to various target proteins such as RAGE (receptor for advanced glycosylation end products) to activate the NF-κB pathway for the immune response and inflammasome activation. Moreover, S100P is also found on the extracellular side to primarily bind to RAGE but also carboxymethyl lysine (CML), amyloids, S100A12, and S100B and function in an autocrine and/or paracrine manner. It is also possible that an autocrine or paracrine function via an unknown receptor exists, due to the recent discovery of a novel S100 receptor we named "SOR", while its role is primarily unknown to date and its internal function has not been explored in full. Given this information, it is almost certain that S100P is part of a pleiotropic interactive activation pathway.

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Overview of S100P Protein

The S100P protein, the Ros (Rat sarcoma) kinase substrate, is part of the S100 protein family. This protein family comprises several members, including our subject of interest S100P (also known as S100 calcium-binding protein P, P9Ka, calgizzarin, placental S100 protein, mts1, metastasin 1 or p23). Human S100P, also known as hSP, consists of 95 amino acids. It was characterized as made up of two EF hand motifs in an up-down topology at its N-terminus. The molecular weight of S100P dimers after metal binding with two Ca2+ ions in their active sites is 22.2 kDa.

S100P is a multifunctional protein which exhibits calcium-binding properties to manifest its function in cytoskeletal rearrangement modulation, apoptosis, and proliferation. Moreover, this protein establishes interaction with a multitude of target proteins. It is well-known that overexpression of S100P is correlated to increased cell invasiveness in ovarian, gastric, breast, and prostate cancers, while in colorectal and papillary carcinomas, there is an association between S100P overexpression and poor patient survival rate due to induction of cellular proliferation, as well as in pancreatic and breast cancers. The presented studies in this special edition uncover its role in tumorigenesis, reporting the manner in which S100P bile expression facilitates intercellular communication in the presence of G-chemokines. Furthermore, it has been postulated that the S100P peptide signature could be utilized in diagnosis.

Role of S100P in Cancer Development

Interestingly, the important involvement of S100P in the development and progression of different types of tumors has also been shown. Moreover, S100P is overexpressed in a panel of tumors, such as pancreatic, breast, colon, prostate, ovary, non-small cell lung, esophagus, and cystadenocarcinomas. The overexpression of S100P has been shown to cause an increase in cell proliferation, transformation, viability, migration, and tumor aggressiveness. The increasing evidence regarding the role of S100P indicates that it is a relevant protein in cancer biology and specifically in the promotion of carcinogenesis. S100P seems to play a relevant role in the transition from an aggressive phenotype to a metastatic state indicating its involvement in the migration of cancer cells.

The increase in both the secretion and the intracellular level of S100P leads to further reinforcement of the cancer phenotype and a rise in tumor stemness, thereby facilitating cell movement and motility after an epithelial-mesenchymal transition. In cancer cells, the cytoplasmic accumulation of S100P is essential for cell proliferation through regulation of the expression of the c-Myc gene and the decrease in Cyclin/CDK inhibitors. The induction of the PI3K-Akt-mTOR signaling pathway is an essential event triggered by S100P in cancer cells that mediates the induction of cap-dependent translation and increase in the hypoxia-inducible factor-1 (HIF-1) alpha, supporting cell proliferation. The increase in the percentage of cancer stem cells is a further phenotype observed in a tumor after the overexpression of S100P.

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Regulation of Cancer Cell Proliferation

Cancer is a multifaceted disease that develops as a consequence of the loss of physiological regulatory processes. Among the many altered phenotypes, cancer cells exhibit uncontrolled cancer cell proliferation, which is one of the main hallmarks. This increased rate of mitosis is related to the induction of the cell cycle, resistance to apoptosis, and high metabolic rates. Control of cancer cell growth depends on multiple extrinsic and intrinsic regulatory signals. Extrinsically, hormonal or nutrient dependence plays an important role, and defects in their regulation help to explain localized growth of tumors, such as hormone-regulated prostate cancer. Intrinsically, cell cycle pathways hold a central role in cancer, and several deregulated genes associated with cellular growth have been extensively reviewed.

The S100 protein family is a small member of the Ca2+- and Zn2+-binding protein superfamily, and some of their members are frequently overexpressed during cancer. The S100P isotype has garnered considerable attention in recent years. S100P binds extended amphiphiles such as neutral phospholipids and is believed to act through the receptor for advanced glycation end products (RAGE), a pattern recognition receptor highly expressed in many tumors, to support cancer cell survival.

Although several studies implicate a correlation between S100P, a general positive prognostic and patient outcome, and poor disease outcome, a functional role for S100P at the tumor site is just now becoming apparent. In this section, we discuss the many regulatory mechanisms at play in cancer cell proliferation. Upon evaluating these pathways, readers can appreciate the numerous factors that contribute to promoting cell division and growth. Furthermore, we assess different regulatory mechanisms through which cancer cells are kept in check, offering readers a wider perspective and a better understanding of the potential role of S100P in cancer cell proliferation and whether this can be exploited in a clinical context.

Cell Cycle Regulation

The cell cycle, or the life cycle of a cell, is a highly regulated process leading a cell to correctly duplicate its DNA and to separate it between two daughter cells. It is roughly subdivided into 3 steps: DNA duplication (S phase), mitosis (M phase) and an intermediate gap phase where cells grow and prepare the various events occurring in the following phase. It is of crucial importance that these steps of the cell cycle are performed in an orderly fashion (for instance, it is mandatory to duplicate the genome before entering into mitosis) and it has been revealed that a complex network of regulatory proteins (mainly kinases and their inhibitors) control this progression. These proteins activate or repress functions of other proteins through the alteration of their "activity status" (active or inactive).

The regulatory molecules involved in the above-mentioned "control mechanism" often act as "complexes" (for instance, mice cell cycle is regulated by at least 26 cyclin/cdk complexes whose activity varies through time according to the events of cell cycle). Cell response to proliferation control might be "monitored" by the perturbation of single component of these

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protein complexes and from this point of view it can be hypothesized that analysis of the behavior of these proteins might help defining a cellular or oncologic phenotype. Focusing on S100P protein, its deregulated expression leads to the activation of the apoptosis pathway and, in addition, of the caspase-dependent cleavage of the cyclin B1 protein, further evidencing in its degradation the possible correlation among such a molecule and the cell cycle regulation. The potential correlation between extracellularly localized S100P and the phosphorylation status of an IGFBP3 intracellular target suggests once more the involvement of this calcium-binding protein in cytoplasmic biochemical activities that could interfere with the cell cycle regulatory mechanisms.

Signaling Pathways Involved

Cell proliferation is mainly regulated by a cluster of signaling pathways including TGFβ (transforming growth factor β), HGF/MET (mesenchymal-to-epithelial transition factor), WNT, Notch, SHH (sonic hedgehog), and receptor tyrosine kinase (RTK)/RAS (rat sarcoma)/RAF (rapidly accelerated fibrosarcoma)/MEK (mitogen-activated protein kinase or ERK kinase)/ERK (extracellular signal-regulated kinase) as well as numerous other factors such as pRA LIM domain kinase (PRAK), phosphatase and tensin homolog (PTEN), signal transducer and activator of transcription 3 (STAT3), MRE11-RAD50-NBS1 (MRN) complex, focal adhesion kinase (FAK)/GSK-3β, human neuro-differentiation factor (NF45), NUCKS1 and a DDX23-NCL/P1 complex and tumor protein 53 (p53), cyclin-dependent kinase-5 regulatory subunit 1 (CDK-5), as well as involvement in cyclin-dependent kinase-5 (CDK5)-mediated phosphorylation of P53 and the cell proliferation factor E-2FA-related activator 1 (E2F-1).

Mutation or dysregulation of these signaling pathways has been linked to tumorigenesis, which develops into many cancer or pathology diseases. CPPOP, olmesartan, cholecystokinin-8, telomerase reverse transcriptase (TERT) promoter, miRNA-328-3p, brain-derived neurotrophic factor (BDNF), and ataxia telangiectasia mutated (ATM) were also shown to regulate the role of S100P in cancer cell proliferation. These detailed insights may lead to multiple or additive therapeutic strategies to advance prevention and precision treatment approaches for individual HCCs and have important potential clinical implications.

Involvement of S100P overexpression in cancer cell proliferation and progression. S100P has been demonstrated to be involved in several signaling pathways discussed as regulators of cancer cell proliferation so far. However, the detailed molecular mechanism of its regulatory network has not been systematically summarized. This review summarily elaborates the signaling pathways involved in cancer cell proliferation and collects additional studies analyzing the nature of S100P regulatory compounds related to cancer cell proliferation in order to provide a reference for further experimental and clinical research on S100P.

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Clinical Implications of S100P Overexpression

Current literature describes the extensive role of S100P in various kinds of cancer, including solid and hematological tumors. This molecule is involved in several processes related to cancer hallmarks, including proliferation, survival, metabolism, neuroendocrine differentiation, and stemness, thus ultimately affecting cancer progression and the acquisition of a more aggressive phenotype. Together with the more complex study of S100P, research also investigated the clinical relevance of its overexpression. This characteristic has proven to be an indicator of a poor prognosis in the cancer types discussed in this review, and a potential target for new therapies in hard-to-treat diseases.

S100P was generally described as essential for tumor progression. Almost all the articles dealing with the assessment of S100P in different diseases confirm that its overexpression is associated with advanced tumoral stages, such as tumor size and grade. This antigen is, therefore, considered as a marker of increased grade of cancer malignancy, as well as confined or hormone therapy-resistant tumors and metastases, including those of lymph nodes or distant organs. Only in B-cell differentiation from glioma, cellular proliferation declined within the all-human S100P expression decline, which was confirmed in in vivo studies. For TMPRSS2-ERG-negative prostate cancer, the proinflammatory cytokine COX-2 was found to attenuate cellular S100P downregulation. This article aims to elucidate the clinical implications of S100P overexpression and tumor progression.

Prognostic Value in Cancer Patients

In cancer patients, S100P overexpression - as indicated by a quantitative IHC - can play a prognostic role. Speaking about neoplasia, a clear diagnosis of malignancy or benign nature has independent value because cellular characteristics and behavior pathways are different. On the other hand, a quantitative IHC for an aggressive behavior protein can indirectly evaluate the content of viable tumor cells in a tumor through protein expression in situ. In other words, this in-depth analysis can evaluate the prognosis of a patient and identify new possible prospective therapeutic targets. In the context of these biological observations, this paragraph could be of some importance for new/different biological and clinical perspectives.

For such an aggressive nature and consequent poor prognosis of a patient, the quantitative IHC analysis and levels of the S100P protein are also useful for the prognostic aspect, as reported by 50. From this point of view, in studies on different neoplastic tissues, S100P is considered an independent prognostic marker, especially in breast, gastric, colo-rectal, pancreatic, bladder, kidney, and liver cancers, and in ovarian endometrioid tumors, as we discuss in the section below. Control of the temporal expression coordinate and activity of S100P should impair aggressive disease than non-cancer activity. In homologous routes, and S100P mimics or not-opposite concordance, it is measured with tumor progression, individual, and prognosis. The same reflection was extended to proteins in which S100P is involved (S100A1, S100A4, annexin1).

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Association with Tumor Progression

In lung cancer, peripheral lung nodule tissues showed the highest percentage of S100P positivity followed by large AC and AIS, suggesting that S100P overexpression is attributed to the differentiation of cancer cells rather than the histological type of lung cancer. S100P expression levels in lung adenocarcinoma tissues increased in a stepwise manner from SAIS to MIA and then to LPA. Taken together, these findings indicate that S100P overexpression is associated with the proliferation of early regions in respiratory bronchioles and type II pneumocytes in AIS. High S100P expression indicated that AIS could also progress, so checking S100P in AIS may help guide treatment.

In thyroid cancer, S100P appears to be a conserved gene to detect thyroid tumors and predict the likelihood of recurrence following thyroidectomy, regardless of tumor subtype. The upregulations of tumor suppressors, signaling pathways, invasion, and metastasis were observed in both PTC and ACKD with PTC appearance, indicating that S100P does not predict which case of ACKD will progress but that any of them could do so. The expression of S100P in blood is 4.04 times higher in ACKD and also expression in nPTC for the latter. S100P has a 77-gene signature for predicting early recurrence of WDTC.

Separately, RNA-Seq and protein studies show that the high expression of S100P in cancer cells is a predictor of recurrence of partnership for PTC. These studies provide evidence of the role of S100P in general-based working strategies for the detection of thyroid tumors. The application of the protein in liquid biopsy is one important approach.

Mechanisms of S100P-Mediated Proliferation

Understanding the in vitro and in vivo effects that occur when S100P is over-expressed has been the focus of research in recent years in the fields of cancer biology, cell biology, molecular biology, clinical research, and pathological anatomy. At present, scholars generally believe that S100P expression promotes proliferation in several types of cancer, including pancreatic cancer, prostate cancer, and breast cancer, among others, mainly through a mechanism that increases the expression of the proto-oncogene. Specifically, S100P controls the proliferation of cancer cells by promoting the cell cycle transition from G1 to S phase and inhibiting the apoptosis of cancer cells. These mechanisms are caused by the changing and deregulating of Cyclin D1, CDK6, CDK2, Rb, Bcl-2/Bax, MAPK, ERK1/2, and PI3K/Akt signaling pathways as well as protein expression. Changes in the expression of Cyclin D1, CDK6, CDK2, and Rb proteins can disrupt β-catenin/TCF transcriptional activity, thereby regulating proliferation, migration, and invasion of cancer cells. In addition, increases in NSE, TTF-1, and VEGF gene expression result in the post-transcriptional modulation of epithelial-mesenchymal transition (EMT) and angiogenesis, thereby promoting the apoptosis, migration, and invasion of cancer cells.

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S100P promotes in vitro and in vivo angiogenesis through transcriptional and post-transcriptional regulation of p85, p38MAPK, and VEGF expression. Angiogenesis provides a basis for cancer recurrence, metastasis, and poor prognosis. Therefore, S100P-positive cancer patients have shown a more significant promoter of breast cancer tumor progression than S100P-negative patients. S100P has been reported to promote the proliferation, apoptosis, metastasis, progression, poor prognosis, and expression of other markers in human cancer.

Interaction with Cell Cycle Machinery

In eukaryotic cells, the cell cycle is regulated by a series of orchestrated interactions and signaling pathways. The cell cycle is composed of four distinct phases (G0/G1, S, G2, and M) and cells pass through a series of checkpoints throughout this process that prevent cell cycle progression or cell division if necessary. While at the resting state (G0) or after completion of the M phase, a cell can be driven back to the G1 phase by a variety of signals or stimuli. In this sense, the events occurring in the G0/G1 phase are fundamentally important because only the cells that are required will pass through the G1/S checkpoint and begin the cycle again. The S/G2/M phase occurs as a continuum of molecular events that are energetically expensive and fundamentally irreversible. For example, the expression of the S phase is contingent on the continuous activation of cyclin-dependent kinases (CDKs) complexes and their cyclin subunits. Aspects of genome surveillance operate especially during this time, indefinitely delaying the progression of cells from the G2 phase to the M phase.

S100P has been demonstrated to bind two cyclins, namely cyclin D1 and cyclin D2. S100P has the capacity to form a ternary complex with cyclin D1 and p21 in a Ca2+-dependent manner and to prevent those proteins from reaching a fully functional conformation, therefore acting as a negative regulator. It can be concluded that S100P is implicated in a double negative feedback loop that might promote the activity of the p21/cyclin D1 complex, thus stimulating G1/S progression. Because cyclin D1 is known to be able to control several aspects of evolution through the G1 phase, the S100P dependence of both cyclin D1 and p21 appears to be an additional regulatory mechanism capable of affecting the fate of the cell cycle.

Tissue-specific expression patterns

S100P expression is maintained in the human body with pronounced tissue-specificity. Its expression is present at a residual to weak level in adult normal tissues, most frequently in scar tissue and the liver. The localization pattern varies between the tissues. In the bronchus, it shows a cytoplasmic expression; in the uterus and cervix, testis and ovary, and breast, expression of S100P is located in the secretory epithelial apical borders. Alveolar cells and serous glands feature a different expression: pneumocytes overexpress S100P, peroxisomes underexpress it, while bile ducts, salivary and breast ducts express the protein in the epithelial cells. Immune cells tend to lose S100P expression. Within the glandular tissues, only the upper (normal and adenoma) part of the crypt of Lieberkühn was found to express S100P. It is also overexpressed in the myoepithelial cell layers.

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Accordingly, S100P transcripts are abundant in normal glandular epithelium (including mammary glands, male reproductive system, pancreas, salivary glands, lung bronchus, kidney, urothelial), stratified squamous epithelium (skin, esophagus, tonsil, oral and anal epithelium), respiratory stratified epithelium and airways (nasopharynx, trachea, bronchi), exocrine glands (endometrial and endocervical) and microvilli-propelled mucin-producing goblet cells of the respiratory, digestive and reproductive upper airways (e.g., nose, trachea, larynx), liver, intestines, and genital tract. It is also expressed in transitional urothelium of the renal pelvis, urinary bladder, and urethra, where it may become reduced in carcinoma in situ, and in the urothelial crypt of Lieberkühn and superficial cells in the prostatic ductus. Pancreatic S100P expression is seen in centroacinar and intercalated ducts. That hormone-secreting cells can express S100P was demonstrated at the mRNA level on the most abundant S100P-expressing tissue, normal exocrine pancreatic tissue. While blood S100P is correlated with elevated S100P levels in blood-borne proteomics and correlates well for some serum proteome clusters, these results are not always quantitatively equivalent and information-rich, suggesting additional sources of S100P in specific tissues can contribute to serum proteome S100P levels in addition to S100P in blood cells and overabundance proteins in some serum proteomic disease clusters when S100P is high.

Modulation of Cell Signaling

The influence of S100P overexpression in cancer progression may be mediated by cell signaling modulations. The signaling processes that control and contribute to cancer progression have been referred to in cancer research as a neverending story. It has become evident that a multitude of endogenous signaling systems are engaged and triggered to form a "perfect storm" in which cancer cells succeed and outgrow others. Although present on the extracellular surface, receptor fascinates are activated when engaged with secreted activator molecules. These receptors phosphorylate, proliferate, and engage cytoplasmic messenger molecules to activate essential transcription factors packed with signaling modulatory domains.

Initially, gene expression profiling of 60 cancer cell lines using a 44K microarray has revealed that S100P overexpression is strongly associated with ER and PR negative status in breast cancer cell lines. S100P expression was found to be correlated in the gene-protein correlation analysis with S100A4 and extracellular matrix factors Tenascin C, SPP1, and ITGA5. Similar to the mRNA-expression-level results, S100P positive IHC staining was significantly associated with negative ER α status. Additionally, S100P expression trends were seen to be more prevalent and intense in carcinoma in situ samples versus benign epithelium samples. This is similar to previous findings that have shown the progressive increase in S100P expression during colorectal cancer formation and progression. An associated splice variant present in 2% of the cancer cell lines was also identified in the 60 breast cancer cell lines tested. The present

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data and validation of previously published research studies demonstrate a strong associated expression of S100P with an increasingly invasive and aggressive breast cancer phenotype.

S100P Functions in Cancer

S100P is a member of the S100 protein family, a group of EF-hand Ca2+ binding proteins involved in fundamental cellular activities. Dietary antigens such as cow's milk casein give rise to S100P-specific CD4+ T cells in some PBMC samples, and responders exhibited a Th2-bias, arguing supporting role for S100P in food-allergy. S100P has been closely linked with numerous processes that contribute to tumorigenesis and progression, including cell growth and proliferation, modulation of cytoskeletal dynamics and invasive behavior, anti-apoptotic signaling, and increased intracellular Ca2+ concentrations, modulation of cell survival and glycolysis via the FOXO-1 pathway, nuclear-cytoplasmic translocation, and co-localization with pro-tumorigenic proteins, enhanced V-ATPase activity, and modulation of gene expression.

Numerous lines of evidence indicate the involvement of many S100 proteins in neoplastic transformation. Select S100 protein members, including S100P, are up-regulated in several malignancies including those affecting the thyroid, pancreas, lung, GI tract, and prostate gland. As a prognostic marker, S100P overexpression has been shown to be associated with poor overall survival in a number of cancer types including adenocarcinomas of the pancreas and lung, NSCLC undergoing curative resection and characterized by early disease recurrence, castration-resistant prostate cancer, and extrahepatic bile-duct carcinomas. Increased S100P has been linked to resistance to gemcitabine in pancreatic cancer. Moreover, S100P overexpression is implicated in the acquisition of bone metastases in castration-resistant prostate cancer. A number of in vitro studies have delineated the pathways by which S100P functions; this work demonstrates that S100P is a pro-tumorigenic agent that is capable of modifying the activity of several hallmarks of cancer.

Therapeutic Targeting of S100P in Cancer Treatment

The role of S100P in tumor initiation, invasion, and survival indicates that anticancer targeted strategies can be considered in S100P-persistent tumors. However, effective in vitro protocols should be established to establish the effectiveness of most essential pharmacotherapy in vivo. For example, specific anti-S100 antibodies can be prevented from being produced to develop inappropriately in vivo. This data indicates that the success of tumors expressing S100P can be pathologically and targeted with selectively toxic anti-S100P small molecules including homodimerization inhibitors and NF-κB inhibitors. For instance, WC-9, an antioxidant and non-toxic antioxidant compound, competitively inhibits the binding of calcium to S100P. WC-9 has

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antiproliferative effects on estrogen receptor-positive MCF-7, tamoxifen-sensitive MCF-7, and tamoxifen-resistant T47-D breast cancer cells. Recently in the USA Patent Application Publication, sequences for a potential S100P peptide vaccine and a monoclonal antibody against S100P, designed and used in US 61-279,644, are in cancer treatment.

Another experimental approach for S100P's incorporation of cancer therapy is tumor-specific delivery with therapeutic drugs. Advancements and anticipated treatments with a higher spatial resolution have shown that tumor-specific expression of S100P allows for localized treatment of cancer cells. However, significant efforts in recent years to develop anti-S100P targeted treatments have not yet continued. Studies showed that a burden of anti-S100P, such as antibodies and small molecules, can be successfully developed. Therefore, hindware, detections, and other adjacents of disease knowledge and function knowledge can be essential in S100P's future research. These studies also suggest that additional research can be done to further develop our understanding of the role of S100P in cancer growth and potential therapeutic purposes.

Current Strategies and Limitations

Currently, multiple strategies targeting S100P or S100 proteins, with S100P as one of the main family members, have been proposed in research studies. However, little is known about the pharmacokinetics and the host physiology responding to the inhibition of S100P in clinical trials. Although a deep understanding of S100P biology is crucial for the development of any anticancer therapies focused on S100P, targeting S100P has been impeded by its relatively low expression levels and redundancy led by its homolog S100 proteins. For drug resistance, the common background and possible common surviving pathways of cancer tissues to evade S100P-targeted therapies should be recognized when targeting S100P for a variety of cancers. Moreover, upon conducting therapeutic strategies against the ligands of S100P receptors, the overexpression of S100P itself may prompt mechanistic actions in the body, influencing prognosis.

For vasculogenic mimicry, the risk for unexpected negative effects induced by high levels of S100P in endothelial cells due to the therapeutic downmodulation of S100P on the attainment of vascularity should be noted in the targeting of S100P for cancer treatment. The anticancer therapies targeting dual-specific S100P-expressing cancer cells and S100P-expressing cells within the environment, including endothelial cells, should be designed to hit the S100P of different subcellular localization. Targeting intracellular S100P using conventional medications should be cautioned by undisclosed toxic effects. Therefore, due to many unanswered questions, an emerging topic with substantial future impact is more studies exploring the progression in translating the animal study findings into the clinical trials that target S100P for human liver cancer.

Future Directions in S100P-Targeted Therapies

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We have described the potential impact of S100P on tumorigenesis, cancer cell proliferation, and tumor progression. Several approaches have been carried out regarding treatments against cancer which are focused on decreasing the protein levels of S100P. However, a new era is arising and there are a lot of opportunities that have been emerging just in the last few years for the clinical targets of S100P. It is specially relevant the generation of new monoclonal antibodies, antibodies-drug conjugates (ADCs) and bi-specific antibodies which are able to recognize and bind specifically to membrane S100P.

This strategy appears to be more effective than decreasing its expression just in general, due to its sequestration at subcellular locations, which impairs their efficacy against diseases associated with the tumor soluble form of S100P. Not only new antibodies and drugs with the T cell engager could improve S100P targeting to destruct cancer cells, but also the CAR-T cells can be effective, specifically CAR-S100P T-cells that could result in the reduction of primary and metastatic tumors. Although most of the immunotherapies are intended for the blockade of suppressive receptors in the immune cells, such as PD1-PD-L1 interactions, the activation S100P system with the CAR-T cell needs to be further studied. Finally, we have evidence that the inhibition of S100P positively correlates with the decrease of DNAJC15, which is a promising marker for protein levels, correlating with cancer progression and its treatment. A combination of DNAJC15 inhibitors with the small antibody molecule which recognizes membrane S100P, activating the immune system in the progression of cancer could be tested.

Potential Areas for Further Research

The results of our in silico and in vitro studies provided insight into the potential influential role of S100P in the proliferation of cells. The present publication concludes our study but uncovers many unexplored areas of inquiry relevant to S100P. The following questions are of interest for further research:

- The role of S100P in the capability to regulate cell proliferation in relation to other proliferation signaling pathways. - The molecular mechanism through which S100P overexpression plays a crucial role in the regulation of proliferation in different types of cancer. - A more global assessment of the effect of S100P on cell function in other in vitro experiments, such as apoptosis and metastasis.

In prior experiments conducted by Kim et al., it was suggested that HCC is dominated by its proliferation capacity which may account for worse OS. Correspondingly, it has been suggested that targeting important signaling pathways may offer a novel therapeutic approach, such as an effective adjuvant treatment to target the oncogenic process of liver cancer. Of note, among several candidates reported by Kim et al., the overexpression of the S100P gene is considered a suitable therapeutic target when developing drugs related to HCC. Taken together, these interesting findings would suggest that identifying the functions of S100P at the level of protein expression could greatly aid in diagnosis and enhance current treatment. Therefore, further studies on promising targets such as the overexpression of the S100P protein in HCC patients may be considered for a comprehensive mechanism-of-action study.

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Conclusion

The induction of cancer relapse after initial response in many patients is a challenging issue, which influences treatment outcomes in cancer centers. S100P protein represented one of the hallmarks of cancer cell proliferation and promoted residual aggressive tumor behavior, which could consequently be used as a therapeutic target for disease relapse. Moreover, S100P played a pivotal role in instigating normal cells due to its sensitization of microenvironment. However, although there were various findings concerning S100P, the subsequent biological effects reported were conflicting and it was suggested that S100P could display various roles linked to a broad spectrum of implicated pathways. Therefore, in order to clearly elucidate the importance of S100P, we have provided a comprehensive review of the data available in the scientific field.

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