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REVIEW ARTICLE

Possible Relation between CD9 and acute leukemia: A Review Article

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ABSTRACT

Background: Due to a cascade of mutations that occur throughout the intricate hematopoiesis process, acute leukemia develops. Myeloid and lymphoid cells (B- and T lymphocytes) are the two main cell lineages into which all pluripotent cells in the bone marrow multiply. Nowadays, CD9 can be found expressed in many different kinds of cells and tissues, several cell types, including malignant cells, stromal cells, epithelial cells, endothelial cells, and hematopoietic cells. We intended to outline possible roles in the Possible Relationship between CD9 and acute leukemia. An intriguing strategy to eradicate LSCs and avoid relapses in AML could involve focusing on CD9, this could be accomplished through the utilization of chimeric antigen receptor (CAR) T-cell therapy or monoclonal antibodies. Because CD9 is expressed on monocytes and these cells are involved in AML by generating interleukin 1, reducing their numbers could be an intriguing prospect in this disease.

Keywords: Acute leukemia; CD9; Myeloid cells

INTRODUCTION

Thirteen thousand instances of acute lymphoblastic leukemia (ALL) and thirteen thousand cases of acute myelogenous leukemia (AML) were reported in 2012 in the US, according to the American Cancer Society. Acute leukemia is one of many chronic diseases that emergency physicians (EPs) are seeing an uptick in patients with. A thorough comprehension of leukemia is crucial due to the complexity of the disease and its potentially fatal implications. In low-income societies, poor outcomes and higher mortality linked with leukemia are likely due, in part, to physician-related delays in making the diagnosis [1].

Several mutations occur during the intricate hematopoiesis process, leading to acute leukemia. Myeloid cells (granulocytes, erythrocytes, megakaryocytes, and monocytes) and lymphoid cells (B- and T-lymphocytes) are the two main cell lineages in which all pluripotent cells in bone marrow differentiate. Lymphoid precursors move to lymphoid organs (such as lymph nodes, spleen, and thymus) to finish development, while myeloid cells multiply into mature bone marrow end cells [1].

Cluster of differentiation 9 (CD9), Leukemia-Associated Cell Surface Antigen p24, Motility-Related Protein-1, tetraspanin-29 (TSPAN29)) was initially found in acute lymphoblastic leukemia cells. It is expressed by various organs and cell types, including epithelial, endothelial, stromal, and most cancer cells. Contact between cells, the interaction between cells and the extracellular matrix, migration of cells dependent on integrins, signaling, a fusion of membranes, cell death, inflammation, proliferation, and differentiation are just a few

of the many cellular processes in which CD9 interacts with its various partners [2].

There are two leading roles that CD9 plays in cancer: suppressing tumors and promoting tumorigenic and metastasizing processes. According to recent studies, metastatic cascade events such as primary tumor evasion, intravasation, extravasation, colonization, and tumor growth are all impacted by CD9 expression. CD9 may promote tumor angiogenesis and lymphangiogenesis. [3].

Structure

There are 33 different proteins in the tetraspanin family, one of which is CD9, an integral membrane protein with a molecular weight of 24-27 kDa. Domains EC1 and EC2 of tetraspanins are located in the cytoplasm, and the protein has four segments that span the membrane. It also has one large and one small extracellular loop. There may be a site for N-glycosylation on the 228-amino acid human protein CD9's EC1 domain. The four cysteine residues that comprise the EC2 domain, commonly known as the big extracellular loop, create disulfide bridges (LEL). A Cys-Cys-Gly pattern is highly conserved, and two residues are part of it [4].

Compared to other tetraspanins, the CD9 LEL contains three α-helices that form a stable, conserved area used for tetraspanin dimerization and oligomerization. On the other hand, two other α-helices include a flexible area used for most lateral interactions with other proteins in the membrane. The conserved residues in the transmembrane domains of CD9 mediate additional intramolecular and intermolecular interactions. Tetraspanin protein folding and transport rely on proper transmembrane domain packing and interactions. CD9's amino acid sequence remains primarily unchanged from one species to another. Several biological fluids contain small vesicles (EVs) that contain CD9, which is present in various places, including the nucleus, endocytic compartment, and plasma membrane [5].

CD9 and extracellular membrane vesicles

Both the cell surface and the membrane of EVs include CD9; when extracellular vehicles (EVs) come from multivesicular structures, they are called exosomes; when they come straight from the plasma membrane, they are called exosomes or microvesicles. EVs vary greatly, ranging from approximately 40 nanometers to a few microns, with exosomes being the smallest at 40 to 100 nanometers. Readers may peruse several great reviews for further information on the processes that lead to the creation of exosomes and microvesicles; whether a person is well or sick, EVs play a role in intercellular communication. They transport bioactive materials from the cell membrane and the cytoplasm, including proteins, lipids, and RNAs of diverse sorts. Blood, saliva, tears, seminal fluid, CSF, and malignant ascites are among the many physiological fluids from which EVs can be extracted. The presence of malignancy is frequently accompanied by an increase in the number of EVs associated with a particular fluid [6].

EVs linked to cancer contribute to the formation of the tumor premetastatic niche, an out-of-the-way location that, thanks to neo-angiogenesis, has become an ideal setting for tumor cells to metastasize. The quantity of tetraspanin proteins on EV membranes, including CD63 and CD81, has long been used in traditional approaches for identifying extracellular vesicles (particularly exosomes) in various body fluids. On top of that, they could have a role in cellular uptake of EVs, construction, or composition in general. Here, we demonstrated a significant decrease in EV endocytosis when CD9 is knocked down in EVs generated by breast cancer cells and recipient cells. There is evidence that CD9-positive EVs are crucial in spermatozoa maturation through molecule transfer between epididymal cells and the latter. The eggs of mice secrete EVs that are CD9 positive, which gives them sperm fusion abilities [7].

Cytoplasmic and nuclear CD9

Reports have indicated that CD9 can be found in various locations, including the nucleus, the endocytic compartment, the cell membrane, and

extracellular vesicles. A possible explanation for CD9's presence in both early and late endosomes/multivesicular bodies is that it is released alongside exosomes. One possible explanation for the increased cytoplasmic CD9 levels observed in some tumors—which may indicate heightened malignancy—is an increase in the generation of CD9-positive EVs. Grade 3 epithelial ovarian cancer tumors have CD9 moving from the plasma membrane to the cytoplasm, unlike grade 1 tumors. Furthermore, it was demonstrated that CD9 expression was minimal in tumors and metastases with high grades, indicating a potential negative relationship between tumor grade and CD9 expression. There was a higher effect of CD9 expression on disease-free survival in the subgroup of patients with head and neck squamous cell carcinoma who had both cytoplasmic and membranous patterns compared to the cohort whose pattern was solely membranous. We discovered that CD9 is present in both the cytoplasm and the nuclei of cells, as seen in specimens from patients with primary ductal breast cancer [8].

CD9 and neoplastic disease

Only recently, multiple cancer models demonstrated CD9's pro-tumorigenic and pro-metastatic action despite its original classification as a tumor suppressor. These varied and often contradictory roles are seen in different cell types, especially in transformed cells; CD9's expression levels within TEMs and its interactions with other proteins could lighten this mystery [9].

CD9 as a regulator of tumor development and progression

Several research have found a correlation between CD9 expression levels and cancer aggressiveness. These studies have used immunohistochemistry and patient prognosis as their bases, and they have also used tumor cell lines and animal models to modify CD9 levels. Declining CD9 levels in prostate cancer patients are associated with cancer progression, possibly as a result of deletions or mutations in the protein's transcript. This trend is particularly noticeable in patients with advanced disease, suggesting that CD9 inactivation may be a critical factor in the progression of this malignancy. Prostate cancer rates are on the rise. People who were taking androgens showed signs of CD9 expression. To investigate the function of endogenous CD9 in prostate cancer initiation and progression, in another study, they crossed CD9-knockout mice with TRAMP (transgenic adenocarcinoma of mouse prostate) mice, a model of spontaneously developing and metastasizing prostate cancer. After CD9 deletion, liver metastasis was significantly worse, indicating that CD9 inhibits the course of the disease [2].

Several studies in lung cancer patients have found evidence of a CD9-induced decrease in tumor growth and progression, but the molecular mechanism(s) underlying this impact has not been investigated. Two main types of lung cancer are NSCLC and HLN, the latter of which is characterized by highly aggressive small cell lung cancer (SCLC). Because their cancers metastasize so early, SCLC patients have a dismal prognosis compared to NSCLC patients. The effects observed—i.e., the reduction of lymph node metastasis—when CD9 was overexpressed in epidermoid Lewis lung carcinoma cells were shown to be due to limiting cell motility, according to an orthotopic NSCLC model. Nevertheless, the implantation site continued to grow unabated despite this overexpression. The MAC10 human lung adenocarcinoma cell line showed cell motility and tumor growth inhibition suppression due to CD9 ectopic expression; the extent to which this impact was observed was level-dependent.[10].

In contrast to less aggressive non-small cell lung cancer (NSCLC) lines, the majority of SCLC lines examined by Funakoshi et al. [11] exhibited low or non-existent CD9 expression, and the ectopic expression of CD9 inhibited the integrin β1-dependent motility. Overexpression of CD9 ectopically increased the likelihood of apoptotic cell death in SCLC cells, whereas CD9-negative cells exhibited better post-adhesive morphological differentiation,

survival, and matrix metalloprotease-2 (MMP-2) production via the phosphoinositide 3-kinase (PI3K)/Akt pathway.

There was a negative correlation between clinical stage and CD9 expression in tumor tissues of breast cancer patients with ductal carcinoma. Metastatic lymph nodes showed lower levels of CD9 expression compared to primary breast malignancies. Patients with high breast malignancies had substantially better overall and disease-free survival rates compared to patients with common breast cancers. An even more compelling indicator of disease-free life than estrogen receptor, tumor, or lymph node status was CD9 positivity [12].

While lymph node and pathological status are associated with CD9 gene expression in pancreatic adenocarcinomas, histological grade, and CD9 gene expression are inversely related. Multivariate research revealed that CD9 status, along with CD82 transcripts, was the most significant factor, and patients whose tumors had a reduced CD9 mRNA level had a poorer survival rate than patients whose tumors were CD9-positive. Patients with malignant mesothelioma who expressed CD9 had a better prognosis [13].

CD9 as a cancer promotor

Additionally, CD9's pro-tumorigenic action was aided by specific signaling pathways. By overexpressing CD9 in ovarian cancer tissues and cell lines, the NF-κB signaling pathway was activated, and the pro-inflammatory cytokines IL-6 and IL-8 were inducted. Several possible indicators, including CD9, were overexpressed in a thorough comparison of gene expression between normal ovarian surface epithelium and primary human ovarian cancers [14].

Cancer and tumor angiogenesis target CD9:

There are divalent Abs that work against CD9. However, they have serious adverse effects, such as platelet activation and aggregation. As a result, cancer treatments that target CD9 are still in the early stages of development. To support our claim that targeting CD9 in cancer cell lines and animal tumor xenograft models could be beneficial, we will list studies investigating CD9 [2].

Clinical application of antibodies against CD9

Keep in mind that CD9 on EVs may hurt the use of anti-CD9 Ab at different levels. To begin, these CD9-positive particles, which are nanometer-sized, can neutralize the injected anti-CD9 Abs, both divalent and monovalent, and therefore reduce their impact on the target cells. Secondly, it has been suggested that divalent anti-CD9 Ab, as opposed to monovalent anti-CD9-Fab, may enhance the endocytosis and nuclear translocation of CD9-positive cancer EVs in host cells, hence amplifying the pro-metastatic impact of EV-mediated tumor-stroma communication. On the flip side, CD9-positive EVs allow non-cancerous host cells to internalize divalent anti-CD9 Ab, which, when coupled with cytotoxic drugs, might cause injury. In general, EV-related events would have negative consequences and, in extreme cases, be lethal [15].

To summarize, to create novel therapeutic and safety tools against this tetraspanin protein, we must deepen our understanding of CD9 by learning more about its molecular and cellular properties [2].

CD and acute leukemia

Due to the severe nature of acute myeloid leukemia, each patient's chemotherapy treatment plan must be personalized. It is crucial to have new prognostic techniques to improve the prognosis, particularly for individuals with CN-AML. Monitoring medication resistance (MRD) is an intriguing way to determine the efficacy of a treatment. In recent years, much research has focused on using MFC to detect markers produced in blast cells. However, there has yet to be any success in identifying a cell antigen exclusively expressed in AML, as opposed to physiologic myeloblasts [16].

Touzet et al. [16] found that CD9 is expressed in 40% of AML. Since CD9 is also highly expressed in LSCs, it should be explored while studying these cells using the MRD technique. It appears that CD9 is involved in leukemic

development and is also connected with cancer stem cell features in ALL [17,18]. It is worth noting that CD9 antigen expression is absent from normal HSCs but is present in AML blast cells and LSCs. Because most LSC-associated antigens (such as TIM3, CLL1, and CD244) are coexpressed on normal HSCs and progenitors and expressed at lower levels on these cells than on bulk cells, these results are significant [18].

An intriguing finding in a recent study by Coustan-Smith et al. [19] Around 30% of AML cases, unlike normal myeloblasts, an upregulation of CD9 on blast cells was identified during MRD monitoring in AML by MFC. Also, their results showed that CD9 was abnormally overexpressed on CD34+CD38− AML cells during diagnosis and relapse in 10 matched samples, which supported the use of the MFC technique on LSCs. They examined CD9 expression on progenitors from 17 healthy BM and 17 malignant BM. Therefore, further research with both standard and malignant samples must confirm their findings about LSCs [16].

Based on these first findings, attempting to remove LSCs and prevent relapses in AML by targeting CD9 with monoclonal antibodies or chimeric antigen receptor (CAR) T-cell treatment is an intriguing strategy. Anti-CD9 has previously been tested in lab settings and can potentially cause Jurkat cells to die and B-ALL cells to stop dividing. Depletion of CD9 cells, which are expressed on monocytes and are involved in the evolution of AML through the production of interleukin-1, could be an intriguing aspect of this disease [20].

The study conducted by Touzet et al. [16] showed that CD9 had a positive impact on AML prognosis, particularly on EFS and RFS, in both univariate and multivariate analyses. Although the MFI values did not emphasize this beneficial function, it was associated with the fraction of CD9+ blast cells. A few patients in this group were found to be CD9 positive, even though their expression was weaker than granulocytes. On the flip side, several patients' blast cells had a robust CD9 MFI, but the tiny sample size meant they were classified as CD9 negative. Thus, there is no direct correlation between RFS or EFS and the density of CD9 antigen at the cell surface. Nonetheless, CD9 expression on blast membranes likely indicates alterations in the cellular characteristics of these cells. Research needs to be done into how this expression contributes to improved chemosensitivity (This chemosensitivity may be associated with the involvement of tetraspanin molecules in several cellular activities, such as cell domiciliation or quiescence).

We examined three datasets of LSC RNA sequencing and one dataset of AML MRD microarrays to find a more precise marker of AML LSCs. One of the potential markers for AML LSCs is CD9, the most highly elevated membrane molecule. CD9 has been implicated in various forms of CSCs, such as those found in pancreatic, breast, ovarian, glioma, and B-acute lymphoblastic leukemia [21].

Potentially, better patient care and prognosis can result from a better understanding of the mechanisms that maintain CSCs. During embryogenesis, for instance, there is substantial crosstalk between the Hedgehog (Hh), Notch, and Wnt signaling pathways. The Hh and Notch pathway inhibitors have made great strides in the early stages of clinical testing. They sequenced the RNA of CD9+ and CD9− cells from three AML patients to determine the processes that control the features of CD9+ LSCs [22].

CONCLUSION

An intriguing strategy to eradicate LSCs and avoid relapses in AML involves focusing on CD9, which could be achieved by using monoclonal antibodies or chimeric antigen receptor (CAR) T-cell therapy. In vitro studies have shown that anti-CD9 can suppress the growth of B-ALL cells and induce death in Jurkat cells. In AML, reducing the number of CD9+ monocytes could be intriguing because these cells are involved in developing the disease through their production of interleukin 1.

COMPETING OF INTEREST

The authors reported no potential conflict of interest.

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