

The Role of Ladinin-1 in Cancer

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Abstract. Ladinin-1 (LAD1) is a protein originally called mammalian epidermal basement membrane collagen anchored silk protein. The molecular weight of the protein is 59 kD. It is a cytoskeleton related protein, responsible for maintaining the the coherence of dermal-epidermal junction, and it helps to connect epithelial cells with underlying mesenchymal cells. Biology of cancer includes aberrant cell differentiation and proliferation, unchecked growth, invasion, and metastasis, among other biological traits. LAD1 affects the migration, metastasis, proliferation and other important physiological processes of cells by regulating its expression, thus affecting the genesis and occurrence of cancer. However, the expression of LAD1 in different cancers has tumor specificity. This article reviews the biological functions of LAD1 in different cancers from breast cancer, colorectal cancer, NSCLC, and cervical cancer. It briefly summarizes the structure of LAD1 and further exploration of cancer-related treatment mechanisms can be provided by understanding its potential molecular mechanism of function in cancer cells LAD1 has potential value in anti-cancer treatment because of its important biological functions.

Keywords: Ladinin-1, Cancer, Breast, Colorectal.

1. Introduction

Ladinin 1(LAD1) is a gene sequence located on human chromosome 1, which was first discovered in 1996. Its exon region seems to be conservative in mammals [1,2]. However, The LAD1 gene is not conservative in other vertebrate species and does not exist in invertebrates., which indicates that its evolutionary origin is late. A mouse skin cDNA library was used to clone LAD1. With the exception of the N-terminal extension sequence containing arginine and six tripeptide motifs containing serine, glutamate, and lysine (SEK), the encoded protein has no recognizable domain and can be used as a component of the basement membrane that secretes [3]. In the protein sequence data set, it was found that the sequences between LAD1 and Caldesmon (an actin and calmodulin binding protein) were similar. LAD1 is a protein that has not been characterized so far, and is a regulatory medium for phosphorylation of epidermal growth factor (EGF) to ERK pathway. LAD 1 has been shown to be a mediator of breast cell proliferation and migration in some studies. Under the action of epidermal growth factor, LAD 1 is post transcriptionally induced phosphorylation, partially co located with actin stress fibers, is a fibroin binding regulator of actin dynamics, and a marker of invasive breast cancer [4]. LAD1 was found to be expressed in several epithelial organs, such as kidney, as well as transcribed in cancerous cells analyzed using GeneCards and cBioPortal (www.cBioPortal.org/index.do). LAD1 expression protein participates in the formation of basement membrane and plays a very important role in maintaining the structural stability between epithelial cells and extracellular matrix. Ultrastructural analysis found that LAD1 is a new protein that can connect anchoring filaments, and it is mainly expressed on the basement membrane below the hemidesmosome [5], which plays an anchoring role between cells and extracellular matrix (ECM).

Increased cell proliferation, invasion and migration are related to tumor metastasis, which is the primary reason for the disease's intractability [6]. Cell proliferation, invasion and migration depend essentially on the mechanical movement of cytoskeleton proteins, which is regulated by the

interaction and signal of macromolecular complexes ECM. These macromolecules adhere by combining integrin heterodimer and adapter complexes (e.g., talus, fibronin and tensin) with extracellular domains. The adapter complex captures the retrograde flow of actin filament (F-actin). This interaction array of ECM, integrin, adapter and F-actin generates the traction force of cell movement [7]. Relevant studies have shown that LAD1 forms a complex with F-actin, which affects the phosphorylation expression of downstream ERK and AKT, thus, affecting the proliferation and migration of cancer cells, invasion and apoptosis of tumor cells.

With regard to the function of LAD1, people only know that the dermal epidermal junction is maintained by this protein, and there is expression deletion in the tissues of patients with borderline bullous epidermolysis [8]. However, in the next 20 years, there was no progress in the research on LAD1 and human diseases. In 2015, some scholars found that when using gene chips to study the animal models of papillary thyroid cancer, they found that 7 genes, including LAD1 and RASA1, were specifically expressed in BRAF V600E (mutant) thyroid cancer [8]. Later, Klobucar M et al. found that LAD1 was highly expressed in laryngeal squamous cell carcinoma and invasive breast cancer compared with adjacent tissues by using proteomic methods [8], and therefore believed that LAD1 might promote tumor occurrence as a potential oncogene, but the evidence was insufficient. IngaPeters et al. found that hypermethylation of LAD1 promoter region indicates a short survival period of renal cancer patients [8]. LAD1 may have a specific role in tumors, based on these results.

Studies have shown that LAD1 is expressed in basal cell like and triple negative breast cancers, suggesting that the expression of LAD1 in breast cancer may be associated with a poor outcome for patients [8]. In the study of thyroid cancer, LAD1 gene was found to be specifically expressed in tumors with BRAF V600E mutation [8]. It has also been reported that LAD1 is highly expressed in squamous cell carcinoma of the mouth and laryngeal carcinoma, and plays a role in tumor metastasis, suggesting that several factors play a role in the occurrence and development of tumors, including LAD1 [8].

2. Breast cancer

Breast symptoms are a result of uncontrolled proliferation of breast epithelial cells in the presence of a variety of carcinogenic factors, and It is the most common malignant tumor in women. Mutations that motility and proliferation induced by growth factors are characteristics of subtypes of aggressive breast cancer. Previous studies have shown that MCF10A is stimulated in culture with EGF by performing analysis of untransformed mammary epithelial cells (MCF10A) using phosphoproteomics. The LAD1 molecule is depleted reduced in animal models, transcription factors associated with cell survival inhibited breast xenograft growth .Further, LAD1 is highly expressed in aggressive subtypes of breast cancer characterized by integration clusters 5 and 10, a atype of cancer that is triple-negative and positive for HER2, Increased LAD1 abundance is a high-grade breast tumor is characterized by these characteristics, which are characteristic of tumors of the breast that are basal-like and HER2-positive in humans.

The signaling pathway activated by EGFR can improve cell migration ability, which has been confirmed in tumor progression studies. Therefore, some studies have used phosphoproteomics technology to analyze the EGF stimulated breast cell lines, and found that LAD1 protein is a nessary factor the ERK signaling pathway is activated by the medium EGF, and LAD1 expression is increased and structural modification occurs during this process [8]. Through yeast two-hybrid, proximal ligation and co-immunoprecipitation assays, it was confirmed that LAD1 and actin, another important cytoskeletal protein,proliferation and invasion of breast cells are controlled by them, and they play an important role in cancer progression . LAD1's association with the actin cytoskeleton suggests that EGFR-phosphorylated effectors are involved. Two-compartment cell culture confirmed that the depletion of LAD1 would result mammary cells migrate slower, and further experiments have shown that LAD1 supports migration, adhesion, and extracellular membrane entry in cultured mammary cells.Coprecipitation analysis suggested that LAD1 actin dynamics are regulated by this protein, the

scaffold protein 14-3-3, also known as SFN, may work synergistically with this protein. In breast cancer cell lines, LAD1 binds to filamin and SFN to regulate cytoskeletal rearrangement and participate in signal transduction. LAD1 protein expression is present in the cytoplasm. It appears that LAD1 is involved in actin fiber remodeling in mammary cells, as well as proliferation and motility. LAD1's C-terminal portion physically interacts with FLNAs in mammary cells. FLNAs link actin fibers together and with transmembrane proteins such as specific integrins. Therefore, cellular structure and rigidity will be affected. As well as regulating the G/F actin ratio, FLNA and LAD1 interact to maintain EGF-induced ERK activation, but the specific mechanism of action involved remains unclear. The physical recruitment of SFN to LAD1-FLNA complex may disrupt F-actin-SFN interaction.

Among the members of the MAPK family, ERK, including ERK1/2, P-ERK is the activated form of ERK. Studies have proved that activated ERK plays an critical role in breast cancer development [9,10]. LAD1 can activate ERK signal transduction pathway. LAD1 and P-ERK were weakly expressed in adjacent normal tissues, however, it is highly expressed in intraepithelial ductal carcinoma and invasive ductal carcinoma, and showed an increasing trend. The protein expression of LAD1 and P-ERK was correlated with the histological grade, and the higher the histological grade, the higher the protein expression positive rate. The expression of the two proteins was correlated with clinical way and lymph node metastasis. The optimistic expression rate of the two proteins in stage III and patients with stage IV invasive ductal carcinoma are at higher risk than patients with stages I and II. The expression of both proteins was higher in node-positive patients than in node-negative patients. There was a optimistic correlation between the expression of LAD1 and P-ERK and the expression of LAD1 and p-ERK in invasive ductal carcinoma [3]. LAD1 is highly expressed in breast invasive ductal carcinoma and positively correlated with P-ERK expression. They play a role in regulating breast cancer progression and are associated with prognosis and survival of breast cancer patients. Potential biomarkers in breast cancer diagnosis, treatment and prognosis. There is little research on LAD1, and in-depth study of LAD1 and its upstream and downstream regulators can provide new ideas for target gene therapy of breast cancer.

3. Colorectal cancer

Colorectal cancer, a common malignancy in the gastrointestinal tract, is among the most lethal cancers, approximately 10% of cancer-related fatalities [11]. LAD1 is a colorectal cancer tumor suppressor gene regulated by methylation. The results of Transwell assay and wound healing assay indicated that overexpression of LAD1 significantly inhibited the proliferation, invasion, migration and tumorigenicity of colorectal cancer cells, In colorectal cancer cells, it promoted apoptosis. However, LAD1 did not affect the spontaneous apoptosis of colorectal cancer cells. The results of immunohistochemistry showed that LAD1 was expressed strongly in the cell membrane and cytoplasm of all normal intestinal mucosal epithelial cells, while the global expression of LAD1 was significantly decreased in primary colorectal cancer tissues. Sequencing results expressed that the expression of LAD1 was fell or silenced in colorectal cancer tissues as a result of hypermethylation of its promoter. The co-localization results showed that LAD1 was bound to E3 ubiquitin ligase TRIM21 and LRP6 is a low density lipoprotein receptor-related protein. Statistical results showed that the expression level of LAD1 had no significant difference with gender, age and differentiation, but the low expression of LAD1 was closely related to tumor invasion, lymph node metastasis, distant metastasis and pathological stage. Current research suggests that one of the possible mechanisms of tumor cell resistance to antineoplastic drugs including 5-FU is the overexpression of certain genes. 5-FU is one of the common gastrointestinal chemotherapy drugs. LAD1 overexpression can increase the number of colorectal cancer cell lines, indicating that the killing effect of 5-FU on colorectal cancer cell lines is increased. LAD1 overexpression can effectively increase the drug sensitivity of tumor cells. Colorectal cancer cell lines treated with LAD1 undergo apoptosis. Further investigation of the effect of LAD1 on the cell cycle of colorectal cancer cells showed that LAD1 blocked the G1-

S transition and inhibited cell proliferation in a cell-specific manner. Moreover, overexpression of LAD1 significantly inhibited the tumorigenicity of colorectal cancer cells *in vivo*. Knockout of LAD1 further confirmed the function of LAD1 in inhibiting tumorigenesis *in vivo* [12].

The molecular mechanism of LAD1's biological function in colorectal cancer showed that E3 ubiquitin ligase (TRIM21) was recruited to promote ubiquitination and degradation of low-density lipoprotein associated receptor protein 6 (LRP6) by LAD1. LRP6 is a co-receptor in the Wnt/ β -catenin pathway, thus achieving the inhibition of Wnt/ β -catenin signaling intensity. The abnormality of Wnt/ β -catenin pathway is related to tumorigenesis. At the same time, numerous studies have been conducted, which showed that epithelial-mesenchymal transition (EMT) is an important cause of invasion and metastasis of rectal cancer [13]. The occurrence of EMT in colorectal cancer cells can promote the loss of connection between cancer cells and the basement membrane, and LAD1 happens to be an important structural protein of the basement membrane. Colorectal cancer cells can promote the occurrence of EMT because of their own LAD1 expression silencing. After losing the attachment polarity of basement membrane, the invasive and migratory abilities of cancer cells are enhanced.

These studies have identified LAD1 as a key TSG in CRC, and its biological significance in CRC suggests that it may be possible to diagnose and treat CRC by targeting LAD1.

4. Non-small cell lung cancer (NSCLC)

One of the world's deadliest and most common cancers is lung cancer [14]. The number of deaths exceeds that of breast, prostate and pancreatic cancers, and about 80 to 85 percent are NSCLC [15]. By analyzing the high expression of LAD1 in NSCLC with gene chips, and downloading the survival prognosis curves of Patients with high and low expression of LAD1 from TCGA and Kaplan Meier plot(KMP) database. Survival prognosis differs between patients with high and low LAD1 expression. Additional analyzes showed that LAD1 is highly expressed in NSCLC and that high expression of LAD1 is associated with poor prognosis in NSCLC. LAD1 knockdown inhibits malignant growth, migration and invasion of NSCLC cells, prevents cell cycle progression and promotes apoptosis. In addition, further studies through the detection of protein expression of LAD1 and the change of related protein content after knockdown indicated that LAD1 is a promising prognostic factor and target of NSCLC.

It is generally believed that the increase of cell proliferation, invasion and migration is related to tumor metastasis, and the increase of cell proliferation, invasion and migration essentially affects the mechanical movement of cytoskeletal proteins. The interacting array of ECM, integrins, adaptors, and actin filaments (F-action) generates traction for cell movement. Some studies have shown that LAD1 forms a complex with F-action, which affects the phosphorylation of ERK and AKT, and then affect cancer cell proliferation and migration, affect tumor cell proliferation, invasion and apoptosis.

At present, there are few studies on LAD1 in NSCLC. By extracting NSCLC samples, using the t test method, according to the level of LAD1 classification, its correlation with gender, age, and stage was analyzed in previous study. It is concluded that the high expression of LAD1 in NSCLC was not related to age and gender, but had a significant statistical significance with tumor stage. LAD1 expression varies with tumor stage, with less differentiated tumors showing higher levels of LAD1 positivity.

In summary, the present study identified LAD1 as an oncogene in NSCLC, and revealed the functional mechanism of LAD1 in NSCLC. High expression of LAD1 promotes malignant growth, migration and invasion of NSCLC and is associated with poor prognosis of NSCLC. Pathological analysis showed that LAD1 was not associated with age and gender but was associated with tumor stage. Downregulation of LAD1 inhibits malignant growth, migration and invasion of NSCLC, promoted cell apoptosis through Caspase-3 /PARP1 pathway, and decreased the expression of apoptosis-related protein Bcl2. In addition, related studies have shown that LAD1 knockdown affects AKT, ERK signaling pathway, p-AKT and p-ERK protein expression decreased, leading to Cyclin D1 expression decreased, and then arrested cells in G0/G1 phase by affecting the EMT-related protein

N-cadherin. The expression of Vimentin further regulates the proliferation, migration and invasion of NSCLC. Therefore, future clinical studies on LAD1 are necessary for targeted therapy of NSCLC.

5. Cervical cancer

A malignant tumor of the female reproductive system is called cervical cancer. A growing number of young patients are affected by this disease, which is second only to breast cancer in terms of incidence [16]. It is the goal of cervical cancer research to find new targets for treating the disease. Studies have found that cervical cancer tissues express higher levels of LAD1 mRNA and protein than adjacent tissues. Univariate analysis showed that the expression of LAD1 protein was associated with patient prognosis. In addition, LAD1 was also positively correlated with the expression of other cervical cancer related proteins, such as transmembrane protease serine 4 (TMPRSS4) [17]. Previous studies have shown that TMPRSS4 promotes a variety of biological functions, including tumor growth, metastasis and cell invasion. However, the molecular mechanism of the effect of LAD1 on TMPRSS4 expression is still unclear, and further studies in the future are essential to understand the effect of LAD1 on cervical cancer.

6. Conclusion

In this article, the potential link between LAD1 and cancer development has been illustrated through a brief overview. Current studies have shown that in many cancers, LAD1 exerts its important biological functions by upregulating its expression, while LAD1 overexpression contributes to cancer cell migration and proliferation, thereby affecting cancer initiation and progression; LAD1 enhances cell migration ability and affects tumor migration through phosphorylation of ERK signal transduction pathway. On the other hand, LAD1 interacts with cytoskeleton related proteins to affect cell structure and rigidity, and then affects cell proliferation and movement.

However, in some cancer cells, the opposite situation occurs, and LAD1 often acts as a tumor suppressor gene to assume its important biological function. In addition, LAD1 overexpression can sensitize cells to anticancer drugs and enhance the efficacy of drugs on cancer cells. LAD1 can also affect tumorigenesis by affecting Wnt/ β -catenin pathway. The differences of LAD1 expression in different cancers indicate that the expression of LAD1 is tumor specific, and its effect on cancer cells and the molecular mechanism are not completely the same.

It is unclear how LAD1 causes cervical cancer and there are few studies to support this hypothesis. Current studies only found that LAD1 related Mrna and protein are heavily expressed in cervical cancer cells, and loss of LAD1 expression is negatively correlated with loss of transmembrane protease serine 4 (TMPRSS4) expression. However, the interaction mechanism between LAD1 and TMPRSS4 needs to be further studied.

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