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Research progress of DEAD-box polypeptide 5 in breast cancer

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Abstract: Breast cancer, as a malignant tumor threatening the survival of women in China, its incidence rate is increasing year by year and showing a younger trend, control of cancer cell proliferation, invasion, metastasis is the focus and difficulty of treatment. DEAD-box polypeptide 5 (DDX5), a processing factor regulating RNA biological function, is a multi-dimensional functional protein involved in regulating RNA splicing, ribosome biological function, transcription and translation. It can promote the proliferation, invasion and metastasis of breast cancer through gene amplification, activation of carcinogens, enhancement of epithelial mesenchymal transformation, and reorganization of cytoskeleton. It can also be used as a marker of prognosis and recurrence of breast cancer. This article reviews the role of DDX5 in promoting the proliferation, invasion and metastasis of breast cancer, elaborates its significance in the classification, prognosis and recurrence of breast cancer, and the clinical reports of existing Chinese medicine intervention DDX5 on breast cancer, which can provide a new perspective for the exploration of the etiology of breast cancer and new targeted therapy ideas.

Keywords: DEAD-box polypeptide 5; Breast cancer; Prognosis; Proliferation; Invasion; Transfer; Ginsenoside
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DEAD-box polypeptide 5 (DDX5) is a prototypical member of the DEAD-box family of deconjugating enzymes, also known as P68 proteins. It was identified in studies of immunocross-reactivity by Lane [1] against PAb 204 and was first described as a host protein in 1980. DDX5 can bind to the single and double strands of RNA, provide the energy to unfold the secondary structure of RNA and correct RNA misfolding. DDX5 is involved in clinical disease processes [2], particularly in the promotion of cancer development and carcinogenesis [3]. It acts as a transcriptional co-activator in various cellular deregulation processes such as malignancy, and is involved in the activation of oncogenic transcription factors such as nuclear transcription factor-κB (NF-κB), estrogen receptor α (ERα), β-connexin, androgen receptor, signal transducer factor and signal transducer and activator of transcription 3 (STAT 3). DDX5 is involved the activation of the oncogenic factors, targeting cancer-promoting factors and intervening in tumor proliferation, metastasis, and invasion [4]. When analyzing p68 levels in the same cohort, 141 patients out of 215 cancers (accounting for 65.6 %) showed up-regulation of p68 levels [5]. Subsequently, the high expression of DDX5 in breast cancer cells has attracted more and more scholars' attention [6]. It has been found that DDX5 advances the progression of breast cancer by resisting its proliferation, invasion and metastasis in a variety of pathways [4], while the specific pathways have not yet been fully elucidated. This article reviews the significance of DDX5 in interfering with the biological behaviors of breast cancer. It also reviews its expression in clinical staging, recurrence and prognosis, which is expected to provide a reference for further precision treatment and prevention of disease aggravation.

1 Overview of the structure of DDX5

DDX5 is a nuclear protein with a relative molecular weight of 68,000 that regulates cell growth and division [7]. It is located at the E2F-regulated promoter of genes associated with DNA coding and is involved in the loading of RNA polymerase II related to DNA replication [8]. Its regulatory genes are located on chromosome 17q21, with 13 exons involved in the translation process and homology to the eukaryotic initiation factor 4A (eIF4A) [9]. Multiple genes associated with breast cancer, including *TP53* (17p13), *ErbB2* (17q12) and *BRCA1* (17q21), are all located on chromosome 17 [10].

DEAD-box protein family belongs to the RNA polypeptide superfamily 2 (SF2) with a highly conserved amino acid domain Asp-Glu-Ala-Asp (D-E-A-D). It relies on adenosine triphosphate (ATP) for chain separation and unwinding short double-stranded RNAs [11]. As a member of the DEAD-box family, DDX5 has a core with nine conserved motifs involved in binding of ATP and RNA, cleavage of phosphoanhydride bonds, and polypeptide activity. The polypeptide core consists of two flexibly connected RecA-like domains, D1 and D2,

responsible for ATP binding and recognition of double-strand RNA. The highly conserved motifs Q, I, II, and III constitute the D1 domain, where the Q motif at the N-terminus of the catalytic enzyme center, along with upstream aromatic residues, can regulate ATP binding, affecting the protein affinity for RNA substrates and ultimately regulating polypeptide activity [12]. Animal experiments show that peptide fragments spanning the Q motif of DDX5I can disrupt its cooperation with calmodulin (CaM), thereby inhibiting the cell pseudopod growth, migration, and metastasis [13]. The flexible mechanical movement of pseudopods plays a crucial role in the invasion and spread of cancer cells [14].

The terminal motifs I and II, also known as Walker A (P-loops) and B motifs, are involved in the binding of the α and β phosphate groups of ATP. Motif III is defined by the serine-alanine-threonine (S-A-T) sequence and contributes to the arrangement and activation of the γ-phosphate-modified ATP. Motif III, using the binding energy of γ-PO4 of ATP, coordinates with motifs I, II, and VI, forming high-affinity single-stranded RNA binding sites [15]. The D2 domain at the C-terminus has an RNA recognition domain, primarily composed of motifs IV, V and VI, which interact with the phosphate groups of nucleic acid substrates, providing a basis for RNA substrate specificity [16]. When the D1 and D2 core domains interact with RNA under the influence of ATP, a tightly closed R-loop structure is formed, creating catalytic sites for ATP hydrolysis and RNA binding clefts. This conformational change unfolds RNA and reshapes the corresponding protein complexes, further influencing various cellular processes [17]. An increase in R-loops is associated with the activation of oncogenes or the inactivation of tumor suppressor genes [18]. Whether DDX5 plays a carcinogenic role through R-loop remodeling and its role in breast cancer warrants further exploration.

2 Roles of DDX5 on the biological behaviors of breast cancer cells

DDX5 plays a crucial role in cellular processes involving RNA structure adjustment, participating in the rearrangement of secondary structures in RNA synthesis and selective splicing in transcriptional processes, both closely associated with cancer proliferation, metastasis, and invasion [18]. Initial studies indicate DDX5 amplification in different subtypes of breast cancer, including luminal A, luminal B, ErbB2 and Basal. Knocking out DDX5 reduces the S-phase DNA replication rate by 18.5%, consequently delaying the cell proliferation process [8]. DDX5 can mediate breast cancer cell migration by intervening in the expression of *S100A4* and *LCN2* genes, both of which are target genes of the transcription factor, nuclear factor of activated T cells 5 (NFAT5), which promotes breast cancer cell migration [19]. Therefore, the primary effects of DDX5 on breast cancer cell behaviors are proliferation, invasion and metastasis.

2.1 Promotion of DDX5 for cell proliferation in breast cancer

Normal growth and development require cell proliferation, an essential process involving DNA replication during the interphase of the cell cycle. Aberrant proliferation can lead to various cancers, including breast cancer. DDX5, which is expressed at low levels in normal cells, is essential for organ development and cell proliferation, participating in the transcription of cell proliferation genes [20]. In breast cancer cells, DDX5 often exhibits high levels of expression, which can be attributed to post-translational modifications such as phosphorylation, ubiquitylation, and SUMOylation, as well as its gene amplification and interaction with proteins in relevant signaling pathways in cancer [21].

In mouse experiments, mice with DDX5 phosphorylated at threonine 69 (Thr69) site significantly increased the weight of breast cancer-derived xenograft tumor by 2.5g and the volume by about 5 times, together with optimized Ki-67 staining. This process depends on the mediation of p21-activated kinase 5 (PAK5) [22]. Previous studies have shown that PAK5 accelerates the G1-S transition and promotes breast cancer cell proliferation by activating NF-κB-p65 nuclear translocation to upregulate cyclin D1 [23]. PAK5-mediated DDX5 phosphorylation recruits protein inhibitor of activated STAT1 (PIAS1), promoting SUMOylation of DDX5 at the K53 site to enhance its stability, thus binding DDX5 to the DROSHA/DGCR8 complex, promoting microRNA-10b (miR-10b) processing. MiR-10b is identified as a prospective marker for early-stage breast cancer and is associated with the clonogenic growth of breast cancer stem cells [24- 25].

Analyses of European research data by Mazurek *et al.* [8] reveal 63 amplification sites of DDX5 among 255 breast cancer genomes. In Perou's study [26], genes inhibited by DDX5 knockdown overlap with genes in breast cancer proliferation clusters. Knocking down DDX5 in SK-BR-3 breast cancer cells significantly impairs the expression of cell division cycle 6 (CDC6), CDC45, and minichromosome maintenance deficient 5 (MCM5), essential for DNA replication initiation. Moreover, the binding of RNA polymerase II to gene promoters is also inhibited, resulting in a 5- to 10-fold decrease in cell proliferation. This suggests that DDX5 is essential for breast cancer cell proliferation, and the extent of proliferation depends on the expression activity of DDX5. This provides a new perspective for developing targeted agents for breast cancer with DDX5 amplification.

Among the 63 DDX5-amplified breast cancer genomes, 37 also show amplification of the ErbB2 gene locus. The sensitivity of cells transfected with DDX5mi2008 to trastuzumab, a monoclonal antibody, increases compared to cells carrying an empty vector with DDX5. Targeting DDX5 in combination with trastuzumab presents a new approach for the precision

treatment of ErbB2-positive breast cancer.

DDX5 is involved in the co-activation of the Notch signaling pathway [27]. Notch3 gene amplification is found in ErbB2-negative breast cancer patients. Transfection of Notch3 with siRNA reduces the number of six ErbB2-negative cell lines by about five times and significantly inhibits proliferation. Jagged1 and Jagged2, ligands of Notch3, promote proliferation in ErbB2-negative breast cancer by activating Notch3-CSL signaling pathway [28]. However, recent research by Brahim [29] suggests that Notch3 inhibits proliferation in MDA-MB-231 cells, the triple-negative breast cancer (TNBC) cell line, through the Notch3-HeyL-Mybl2 axis. He *et al.* confirmed that DDX5 activates the Fos-related antigen-1 (Fra-1) pathway, promoting TNBC proliferation [30]. DDX5, a newly identified protein in the Fra-1 pathway, shares 6,699 overlapping gene binding sites with Fra-1 in TNBC, accounting for 62%. This interaction upregulates proliferation-promoting genes including polo-like kinase 1 (PLK1) and CDC20, and downregulates proliferation-inhibiting genes including cyclin-dependent kinase inhibitor 2B (CDKN2B) and CCAAT enhancer binding protein δ (CEBPD), to promote proliferation of Fra-1-dependent TNBC. *In vivo* experiments show that DDX5 deletion impairs origin replication complex 1 (ORC1), ORC6, CDC6, MCM2, MCM5, CDC45 and other genes which replicate DNA precursors to inhibit proliferation. *In vitro* experiments reveal reduced growth and colony-forming ability in TNBC cells lacking DDX5, which enhances the inhibitory effect of Fra-1 deletion on cancer cell growth. This indicates a credible role of DDX5 in promoting breast cancer proliferation, but the specific regulatory pathway remains unclear, and requires further research. Additionally, as a co-transcriptional activator for steroid hormones, DDX5 collaborates with platelet-derived growth factor receptor β (PDGFR β) to regulate the p68-PDGFR-β-AR axis, promoting hormone-dependent proliferation in breast cancer cells [31]. The above studies confirm the pro-proliferative effect of DDX5 in breast cancer proliferation and emphasize the importance of further research to determine the specific regulatory mechanisms.

2.2 Promotion of DDX5 for invasion and metastasis in breast cancer cells

Cancer cell invasion and metastasis involve a complex process involving cell migration and extracellular matrix remodelling. Epithelial-mesenchymal transition (EMT) reduces cell-cell interactions and increases cell motility by converting epithelial cells into mesenchymal cells to confer invasive and metastatic properties on tumors. The conversion of E-cadherin, vimentin and the snail superfamily of zinc-finger transcription factors play a critical role in this process [32]. DDX5 upregulates the expression of the platelet-derived growth factor receptor — PDGFR-β by both *in vivo* and *in vitro* experiments, thereby promoting the expression of the mesenchymal markers, such as vimentin and snail. This downregulates the E-cadherin and promotes EMT, leading to increased migration of breast cancer cells [31].

However, it has long been known that PDGFR-β is expressed in 75% of highly invasive breast cancers and correlates with the invasive phenotype of human breast cancer. The autocrine PDGFR signaling pathway in which it participates promotes transforming growth factor-β (TGF-β)-induced EMT in EpRas cells by over-activating the PDGFR-dependent phosphatidy-linositol 3-kinase (PI3K) signaling pathway. This protects the established mesenchymal phenotype in EpRasXT cells, allowing human breast cancer cells to evade apoptosis and achieve invasion and metastasis [33].

The classic Wnt/β-catenin signaling pathway, triggered by various Wnt ligands, is involved in breast cancer progression [34]. An Indian study [35] found that DDX5 expression is upregulated in breast cancer cells with overexpression of β-catenin, cellular myelocytomatosis viral oncogene (C-Myc) and transcription factor 4 (TCF4). This upregulation leads to increased expression of the EMT markers N-cadherin, vimentin and vascular endothelial growth factor (VEGF). In vivo experiments show that DDX5 induces EMT progression through the Wnt signaling pathway by regulating the nuclear translocation of β-catenin. In addition, MCF7 cells with Wnt3a stabilisation have an approximately 15-fold increase in invasive capacity, establishing a DDX5-mediated β-catenin/TCF4 signaling feedback loop that regulates EMT in breast cancer progression, although the specific mechanism remains unclear. Wang *et al.* [36] found that DDX5 promotes breast cancer metastasis by recruiting Ring1b to form a complex that inhibits E-cadherin expression.

Wang *et al.* [10] innovatively investigated actin dynamics in breast cancer and found that DDX5 promotes breast cancer resistance and invasion through the miR-182-actin cytoskeleton-aton pathway. As a key regulator of miRNA, DDX5 downregulates target cofilin and phosphorylated cofilin by modulating miR-182 in basal breast cancer cells. This leads to the reorganization of the actin cytoskeleton and inhibition of its polymerization, conferring invasive properties on cancer cells.

When DDX5 is knocked down in MDA-MB-231 cells, the expression of miR-21 decreases while the expression of its target tumor suppressor gene, programmed cell death 4 (PDCD4), increases. Loss of PDCD4 expression promotes breast cancer invasion [37]. In addition, DDX5 upregulates the expression of ErbB2 together with miR-21. ErbB2 activation upregulates miR-21, which promotes breast cancer cell invasion [38]. When ErbB2 is knocked out in ErbB2 breast cancer cell lines, DDX5 protein expression is unaffected and vice versa. Therefore, it is speculated that ErbB2-amplified breast cancers with low expression of ErbB2 / high expression of DDX5 may exhibit resistance to

ErbB2-targeted drugs such as lapatinib and trastuzumab. Combining targeted ErbB2 therapy with DDX5 may provide an optimal treatment for ErbB2 breast cancer [10]. In addition, studies have found increased levels of DDX5 in breast cancer patients with lymph node invasion and poor tumor differentiation [6]. In conclusion, DDX5 may promote breast cancer invasion and metastasis through multiple pathways and targets. Determining the regulatory mechanisms of DDX5 and its complex interactions with proteins in related pathways is important for selecting precise targets for breast cancer.

3 The expression and clinical significance of DDX5 in breast cancer tissues

The high incidence and mortality of breast cancer emphasize the importance of precise diagnosis, prognosis, and recurrence in clinical treatment. Despite improvements in breast cancer diagnosis and treatment leading to increased survival rates, the identification of new biomolecules is necessary for more effective therapeutic strategies. The differential expression of DDX5 in various breast cancer subtypes and its correlation with recurrence and prognosis suggest its potential as a novel biomarker of breast cancer.

3.1 DDX5 and breast cancer subtypes

Tumor heterogeneity remains a major challenge in the diagnosis and treatment of breast cancer. The identification of heterogeneous molecules is critical for the development, progression, treatment and prognosis of different breast cancer subtypes. The expression of DDX5 varies between different breast cancer subtypes, making it a molecular marker of heterogeneity. The relative expression of DDX5 increases from luminal to basal phenotypes, with the highest average in basal B-cell lines of 1.17 (including TNBC). This expression is positively correlated with Ki-67 and EGFR [10]. The findings of He [30] are consistent with the above studies and the same BT549 TNBC cells are used. The expression level of DDX5 is highest in BT549 cells, with immunofluorescence quantification values close to 2 compared to luminal A, luminal B and HER2-enriched subtypes. However, the above study does not agree with that of Li [6] who showed that DDX5 was most abundantly expressed in the luminal B type of breast cancer. The study by Mazurek [8] also found that the frequency of DDX5 amplification was highest in the luminal B type, which was 53%. The above differences suggest that DDX5 expression has advantages in determining breast cancer heterogeneity, and the focus is on determining the specific expression of DDX5 that corresponds to each breast cancer type. Interestingly, research by Li [6] also showed that breast cancer patients with elevated DDX5 were associated with ER^-/PR^+ positive status. However, DDX5 also played a role in differentiating between benign and malignant breast tumors, with DDX5 showing strong nuclear staining in malignant breast tumor tissue with a positive rate of 79.4% (54/68) compared to 0 in benign tissue [10]. And in one study, there was no difference in DDX5 expression in six normal breast tissues compared to breast tumors [30].

3.2 DDX5 and Breast Cancer Recurrence and Prognosis

DDX5 is an independent prognostic factor in breast cancer patients. High levels of DDX5 expression shorten overall survival (OS) and disease-free survival (DFS) by approximately 4 years, leading to poorer clinical outcomes [22]. DDX5 has been identified as an effective biomarker for breast cancer recurrence and prognosis. In a study of 868 breast cancer patients, DDX5 was found to be highly expressed in breast tumors compared to surrounding normal tissue, and patients with high DDX5 expression had a very high risk of recurrence (P<0.01). It was significantly correlated with advanced TNM stage, lymph node invasion and poor differentiation (P<0.01), and negatively correlated with recurrence-free survival (RFS) and breast cancer-specific survival (BCSS), associated with shorter survival. Consistent with this, analysis of the correlation between DDX5 expression level and patient prognosis in 801 ER breast cancer patients showed that patients with high DDX5 expression had a poor prognosis (P=0.002), which was negatively correlated with RFS. In addition, breast cancer patients with low levels of ER and DDX5 expression did not correlate with patient prognosis (P=0.51). Analysis of Gene expression-based Outcome for Breast cancer Online (GOBO) showed that distant metastasis-free survival (DMFS) was decreased in the high expression group of DDX5-regulated proliferation-promoting genes (PPG), while it was increased in the high expression group of proliferation-suppressing genes (PSG), which predicted a good prognosis [30]. This highlights the value of DDX5-regulated gene networks in predicting clinical prognosis in breast cancer. However, Liu *et al*. [27] found that DDX5 mRNA expression was decreased in breast invasive carcinoma (BRCA) with tumor progression at T stage, but expression of DDX5 protein was upregulated in breast cancer, further confirming the complex regulatory mechanism of DDX5 in the transcriptional process and the value of DDX5 as a prognostic biomarker in the assessment of tumor is also affirmed.

4 Intervention of Chinese medicine on DDX5 and breast cancer

Ginsenoside Rh1 (G-Rh1), an extract of the Chinese herb ginseng, disrupts the mitochondrial membrane potential, blocks the G1-S phase, and cleaves the activity of caspase-3 to promote apoptosis in TNBC through the accumulation of mitochondrial reactive oxygen species (ROS) [39]. Zhang [40] found that 500 μmol/L of G-Rh1 blocked HER2-positive breast cancer cells in the S phase, induced late apoptosis by experiments *in vitro*, and inhibited the expression of DDX5. DDX5 was positive in 60% (21/35) of HER2-positive breast cancer cells, and

upregulation of DDX5 expression was associated with increased lymph node metastasis and Ki67 levels, later clinical TNM staging, and worse pathohistological grading. It is suggested that G-Rh1 does not affect the expression of the DDX5 gene but promotes the post-transcriptional protein degradation of DDX5 to reverse the malignant biological behavior of HER2-positive breast cancer cells, and it is believed that DDX5 is a prognostic marker and a new therapeutic target for HER2-positive breast cancer. It is worthwhile to investigate the mechanism of G-Rh1 degradation and intervention in DDX5 transcription, given that DDX5 has been found in breast cancer cells for many years. Given the abundance and specificity of DDX5 expression in breast cancer and the possible resistance to targeted therapy in the future, targeted therapy with Chinese medicine interfering with DDX5 may be a new treatment for breast cancer patients. Thus, further research on Chinese medicine interfering with DDX5 provides a direction for future exploration.

5 Conclusion and perspectives

As a potential biomarker for breast cancer, DDX5 plays a driving role in the proliferation, invasion and metastasis of breast cancer, providing new insights for the identification of novel molecular targets and related interventional drug research. It also plays a crucial role in predicting molecular subtypes, prognosis and recurrence of breast cancer, offering a promising molecular targeted therapy for breast cancer patients. Therefore, in-depth research into the specific mechanisms of DDX5 and its interaction with upstream and downstream genes in breast cancer is essential. By exploring the advantages of Chinese medicine in cancer treatment, the combination of Chinese medicine and DDX5 targeted therapy should be considered to seek a better treatment for breast cancer patients.

Conflict of interest: None

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· 研究进展 ·

死盒蛋白 5 在乳腺癌的研究进展

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摘要：乳腺癌作为威胁我国女性生存的恶性肿瘤，其发病率逐年提升并呈年轻化趋势，控制肿瘤细胞的增殖、侵袭、转移是治疗的重点以及难点。死盒蛋白 5（DDX5）作为一种调节 RNA 生物功能的加工因子，是参与调控 RNA 剪切、核糖体生物功能、转录、翻译的多维功能蛋白，可通过基因扩增、激活致癌因子、增强上皮间质转化、重组细胞骨架来促进乳腺癌增殖、侵袭、转移，同时可作为乳腺癌预后与复发的标志物。本文综述了 DDX5 在促进乳腺癌增殖、侵袭、转移中的作用，其对乳腺癌分型、预后、复发的意义以及现有的中药干预 DDX5 影响乳腺癌的临床报道，以期为乳腺癌新的靶向治疗思路提供新角度。

关键词：死盒蛋白 5；乳腺癌；预后；增殖；侵袭；转移；人参皂苷

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Research progress of DEAD-box polypeptide 5 in breast cancer

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Abstract: Breast cancer, as a malignant tumor threatening the survival of women in China, its incidence rate is increasing year by year and showing a younger trend, control of cancer cell proliferation, invasion, metastasis is the focus and difficulty of treatment. DEAD-box polypeptide 5 (DDX5), a processing factor regulating RNA biological function, is a multi-dimensional functional protein involved in regulating RNA splicing, ribosome biological function, transcription and translation. It can promote the proliferation, invasion and metastasis of breast cancer through gene amplification, activation of carcinogens, enhancement of epithelial mesenchymal transformation, and reorganization of cytoskeleton. It can also be used as a marker of prognosis and recurrence of breast cancer. This article reviews the role of DDX5 in promoting the proliferation, invasion and metastasis of breast cancer, its significance in the classification, prognosis and recurrence of breast cancer, and the clinical reports of existing Chinese medicine intervention DDX5 on breast cancer, which can provide a new perspective for the exploration of the etiology of breast cancer and new targeted therapy ideas.

Keywords: DEAD-box helicase 5; Breast cancer; Prognosis; Proliferation; Invasion; Transfer; Ginsenoside

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死盒蛋白 5（DEAD-box polypeptide 5，DDX5）是 DEAD-box 解旋酶家族中原型成员，也被称为 P68 蛋白。1980 年被描述为宿主蛋白，在 Lane 等[1]针对 PAb 204 抗体的免疫交叉反应研究中被发现，在结合 RNA 单双链的同时为 RNA 的二级结构解螺旋提供能量，纠正 RNA 的错误折叠进而发挥正常功能。DDX5 参与临床疾病过程[2]，尤其表现在促进恶性肿瘤发展和致癌作用方面[3]。在恶性肿瘤等多种细胞失控过程中作为转录辅激活因子参与包括核转录因子（nuclear transcription factor-κB，NF-κB）、雌激素受体（estrogen receptor，ER）α、β-连环蛋白、雄激素受体、信号转导因子和转录激活因子 3（signal transducer and activator of transcription 3，STAT3）等致癌转录因子的激活，衔接相应促癌因子，干预肿瘤细胞的增殖、转移、侵袭[4]。在分析同一队列中 p68 水平时，215 例恶性肿瘤患者中有 141 例（占比达 65.6%）显示 p68 水平上调[5]，随后 DDX5 在乳腺癌细胞的高表达，引起越来越多学者的关注[6]，研究发现 DDX5 推进乳腺癌进展表现在多种途径

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控制其增殖、侵袭和转移[4],具体途径尚未清晰。现对 DDX5 在近些年干预乳腺癌生物学行为以及其表达在临床分型、复发、预后的意义进行综述,期望对临床精准治疗以及既病防变提供参考。

1　DDX5 的结构概述

DDX5 是一类调控细胞生长、分裂的相对分子量为 68 000 的核蛋白[7],定位于与 DNA 编码相关基因的 E2F 调节启动子,与 DNA 复制相关的 RNA 聚合酶Ⅱ的加载也需要 DDX5 参与[8],其调控基因定位于染色体 17q21, 参与翻译过程的外显子有 13 个,与翻译起始因子 eIF-4A 有同源性[9]。有趣的是与乳腺癌有关的多个基因包括 *TP53*(17p13)、*ErbB2*(17q12)和 *BRCA1*(17q21)等均处于 17 号染色体上[10]。DEAD-box 家族蛋白是具有高度保守氨基酸结构域 Asp-Glu-Ala-Asp(D-E-A-D)的 RNA 解旋酶超家族 2(SF2)中的一类,依赖三磷酸腺苷实现链分离以解旋短 RNA 双链体[11]。DDX5 是 DEAD-box 家族蛋白的组员,核心含有涉及 ATP 和 RNA 的结合、ATP 磷酸酐键的裂解以及解旋酶活性的 9 个保守基序,解螺旋酶核心由 D1 和 D2 两种灵活连接的 RecA 样结构域组成,分别承担 ATP 结合和 RNA 双链识别的功能。高度保守的 Q-基序及基序Ⅰ、Ⅱ、Ⅲ组成 D1 结构域,位于催化酶活性中心 N-末端的 Q-基序和其上游的芳香残基可调节 ATP 的结合进而直接影响蛋白质对 RNA 底物的亲和力而最终调节解旋酶的活性[12]。有动物实验显示,利用跨越 DDX5IQ 基序的肽片段能够阻断其与钙调蛋白(camodulin, CaM)的合作进而抑制细胞伪足的形成,细胞的迁移和转移成功被阻断[13]。丝状伪足灵活的机械运动对癌细胞侵袭和传播举足轻重[14]。末端基序Ⅰ和Ⅱ,又称为基序 Walker A 和 B,参与 ATP 的 α 和 β 磷酸基团结合,基序Ⅲ根据丝氨酸-丙氨酸-苏氨酸(S-A-T)序列而定义,可参加 ATP 的 γ 磷酸基团的排列和激活,基序Ⅲ利用 ATP 的 γ-PO4 结合能与基序Ⅰ、Ⅱ、Ⅵ协调作用,构建高亲和力的单链 RNA 结合位点[15]。位于 C 末端的结构域 D2 拥有 RNA 识别域,主要由基序Ⅳ、Ⅴ、Ⅵ组成,它们主要与核酸底物磷酸基团相互作用,为 RNA 底物的特异性提供基础[16]。D1 和 D2 核心域当在 ATP 的作用下与 RNA 相互作用时,由彼此分离状态形成致密闭合状态的 R 环,继而形成 ATP 水解的催化位点和 RNA 结合裂缝,通过这种构象改变使 RNA 展开以及相应蛋白质复合物的重塑,进一步影响各种细胞过程[17],而 R 环的增多伴随着原癌基因的激活或者抑癌基因的失活[18],DDX5 通过 R 环重塑是否具有致癌作用以及在乳腺癌的作用机制值得后续研究探索。

2　DDX5 对乳腺癌细胞生物学行为的影响

DDX5 在涉及 RNA 结构调整的细胞过程中发挥重要作用,参与 RNA 二级结构的重排和转录过程的选择性剪切,这两者都与肿瘤的增殖、转移、侵袭密切相关[18]。早期研究显示,在乳腺癌的 luminal A、luminal B、ErbB2 过表达型、基底样型等不同亚型中均出现了 DDX5 的扩增,敲除 DDX5 可将 S 期 DNA 复制率降低 18.5%,从而延迟细胞增殖进程[8]。DDX5 可通过干预 *S100A4* 和 *LCN2* 基因的表达来介导乳腺癌的迁移,上述基因是活化 T 细胞核因子 5 蛋白抗体(nuclear factor of activated T cells 5, NFAT5)转录因子的靶基因,而 NFAT5 参与促进乳腺癌的迁移[19]。因此,DDX5 对乳腺癌细胞行为的影响主要涉及增殖、侵袭以及转移能力。

2.1　DDX5 促进乳腺癌细胞增殖　正常的生长发育过程离不开细胞增殖,DNA 的复制在细胞增殖间期不可或缺。倘若细胞增殖偏离正常轨道,发生异常增殖会导致包括乳腺癌在内的肿瘤。DDX5 在正常细胞中表达较低,是器官发育和细胞增殖所必须的,且参与细胞增殖基因的转录[20],在乳腺癌细胞中常呈现高表达状态,究其原因可能是其翻译后修饰的磷酸化、苏莫酰化和多泛素化,当然也离不开自身基因的扩增以及与相关癌症通路蛋白的相互作用[21]。在小鼠实验中,携带在 Thr69 位点磷酸化的 DDX5 的小鼠乳腺癌移植瘤体增加约 2.5 g,体积增大约 5 倍,明显突出体表,并呈现强 Ki-67 强染色,此过程依赖 p21 活化激酶 5(p21-activated kinase 5, PAK5)的介导[22],而 PAK5 通过激活 NF-κB-p65 核移位来上调周期蛋白 D1 进而加速 G1-S 过渡促进乳腺癌增殖早已被证明[23]。接下来 PAK5 介导的 DDX5 磷酸化通过募集更多的人激活 STAT 蛋白抑制因子 1(protein inhibitor of activated STAT1,PIAS1)来促进 DDX5 在 K53 位点苏莫酰化以提高自身的稳定性,继而促进 DDX5 与 DROSHA/DGCR8 复合物的结合,促进 miR-10b 的成熟,而 miR-10b 被认定为早期乳腺癌的前瞻性标志物[24],与乳腺癌干细胞克隆潜力相关[25]。

Mazurek 等[8]通过分析一项欧洲研究所数据显示,255 个乳腺癌基因组中有 63 个存在 DDX5 的扩增位点。而在 Perou 等[26]的研究中同样发现因 DDX5 敲除被抑制的基因与乳腺癌增殖簇基因存在重叠,于是当敲低 SK-BR-3 乳腺癌细胞中 DDX5 后,DNA 复制启动必需的细胞分裂周期蛋白 6(cell division cycle 6, CDC6)、CDC45 以及微小染色体维持缺陷蛋白 5(minichromosome maintenance deficient 5, MCM5)的表达明显受损伤,RNA 聚合酶Ⅱ与基因启动子的结合也受到抑制,细胞增殖抑制了 5 至 10 倍。然而当敲除缺乏 DDX5 基因扩增的乳腺癌细胞时,细胞增殖抑制不超过 2 倍。这提示 DDX5 是乳腺癌细胞增殖所必须的,且增殖能力大小依赖 DDX5 的表达活性。这为干预 DDX5 的表达活性治疗具有 DDX5 扩增位点类型乳腺癌靶向剂的研制提供了新角度。另外在 63 个 DDX5 扩增的乳腺癌基因组中,37 个也出现了 ErbB2 基因座的扩增,实现了 DDX5 和 ErbB2 基因座的共扩增[8]。当用 DDX5mi2008 转染的细胞与含有 DDX5 的空载体相比时,其对曲妥珠单抗的敏感性增高。靶向 DDX5 联合曲妥珠单抗对于精准治疗 ErbB2 阳性的乳腺癌是一种新思路。

DDX5 参与致癌 Notch 信号的共同激活[27],在 ErbB2 阴性乳腺癌患者发现 Notch3 基因的扩增,用 siRNA 转染 Notch3 后,六个 ErbB2 阴性细胞系数目减少约 5 倍,增殖明显被抑制,Notch3 配体 Jagged1 和 Jagged2 在 Notch3-CSL 信号激活中促进 ErbB2 阴性乳腺癌的增殖[28]。而 Brahim 等[29]最新研究

与之相反,Notch3 则通过 Notch3-HeyL-Mybl2 轴抑制了 MDA-MB231 三阴性乳腺癌(triple-negative breast cancer, TNBC)细胞的增殖,而 DDX5 激活 Fos 相关抗原 1(Fos related antigen-1, Fra-1)通路促进 TNBC 的增殖已被 He 等[30]研究证实,DDX5 作为 Fra-1 通路新识别的蛋白,两者在 TNBC 的重叠基因结合位点达 6 699 个,占比达 62%,通过优先上调增殖促进基因 Polo 样激酶 1(polo like kinese 1, PLK1)、CDC20,下调增殖抑制基因周期蛋白依赖激酶抑制因子 2B(cyclin-dependent kinase inhibitor 2B, CDKN2B)、CCAAT 增强结合子蛋白 δ(CCAAT enhancer binding protein C/EBPδ, CEBPD)促进 Fra-1 依赖的 TNBC 的增殖。体内实验 DDX5 缺失后损害原点识别复合物(origin recognition complex, ORC)1、ORC6、CDC6、MCM2、MCM5 和 CDC45 等复制前体的基因来抑制增殖,体外实验中 DDX5 缺失小鼠的 TNBC 细胞生长和细胞集落能力降低并进一步增强了 Fra-1 缺失对于癌细胞生长的抑制。因此 DDX5 促进乳腺癌增殖的能力是可信的,是否通过 Notch 信号通路影响乳腺癌增殖以及精准机制的确定,亟需相关干预实验验证。另外 DDX5 作为性类固醇激素的协同转录激活剂,可与血小板源性生长因子受体 β(platelet derived growth factor receptor β, PDGFRβ)合作调控 p68-PDGFR-β-AR 轴,推进乳腺癌细胞呈现雄激素依赖性增殖[31]。上述研究可以确定 DDX5 对于乳腺癌增殖的促进作用,但具体调节路径仍存在盲区,进一步的研究显得尤为重要。

2.2　DDX5 *促进乳腺癌细胞侵袭和转移*　肿瘤细胞的侵袭和转移是一个复杂的过程,离不开细胞的迁移和细胞外基质的破坏与重塑。上皮间质转化(epithelial mesenchymal transformation, EMT)通过可塑性的上皮表型的丧失和间充质表型的获得减少细胞间接触以及增加细胞的运动来赋予肿瘤侵袭和转移特性,上皮细胞钙黏蛋白(E-cadherin)、波形蛋白(vimentin),锌指转录因子(snail)的转换调节贯穿始终[32]。DDX5 可同时在体内体外实验中通过上调血小板生长因子受体 PDGFR-β 的表达从而促进间质标记物的 vimentin、snail 的表达,降低上皮标志物 E-cadherin 而增强 EMT,使乳腺癌细胞的迁移增加[31]。然而 PDGFR-β 早就被发现在 75%的高浸润乳腺癌中表达,与人类乳腺癌的侵袭表型相关,其所参与的自分泌 PDGFR 信号通路可促进转化生长因子-β(tubuloglomerular feedback β, TGF-β)诱导的 EpRas 细胞中的 EMT,过度激活依赖 PDGFR 的磷脂酰肌醇 3-激酶(phosphatidylinositol 3-kinase, PI3K)信号靶点,保护 EpRasXT 细胞中已建立的间充质表型,使人乳腺癌细胞系逃避凋亡而达到侵袭和转移的目的[33]。经典 Wnt/β-连环蛋白信号通路由多种 Wnt 配体触发参与乳腺癌发展[34],而一项印度的研究[35]发现 DDX5 可随着 β-catenin、骨髓细胞瘤病毒癌基因(cellular-myelocytomatosis viral oncogene, C-Myc)、转录因子 4(transcription factor 4, TCF4)的过表达而在乳腺癌细胞中表达上调,继而增加 EMT 标志物 N-钙黏蛋白、vimentin 和血管内皮生长因子(vascular endothelial growth factor, VEGF)的表达,体内实验发现 DDX5 诱导 EMT 进展通过 Wnt 信号调控 β-catenin 的核输入来实现。另外含有 Wnt 3a 稳定表达基因的 Wnt 3a-MCF 7 细胞中,细胞侵袭力增强 15 倍左右,构建了调控乳腺癌 EMT 发展的 DDX5/β-catenin/TCF4 信号反馈环,但未阐明其具体转移机制。Wang 等[36]则发现 DDX5 在乳腺癌中通过募集 Ring1b 形成复合物阻遏 E-cadherin 的表达促进转移。

Wang[10]的团队创新性的从肌动蛋白动力学角度出发,发现 DDX5 可通过 miR-182-actin 细胞骨架-eton 通路促乳腺癌的耐药性和侵袭,作为 miRNA 主要调节因子的 DDX5,通过调控基底乳腺癌细胞中 miR-182 来下调标靶丝切蛋白(cofilin)和磷酸化丝切蛋白,致使肌动蛋白的细胞骨架重组并抑制其聚合,赋予癌细胞侵袭特性。当敲低 MDA-MB-231 细胞中 DDX5 时,减少 miR-21 表达的同时上调其标靶肿瘤抑制基因程序性细胞死亡因子 4(programmed cell death 4, PDCD4)的表达,而 PDCD4 表达缺失会促进乳腺癌的侵袭[37]。此外,DDX5 在上调 miR-21 的同时也上调 ErbB2 的表达,ErbB2 也可上调 miR-21 进而促进乳腺癌细胞侵袭[38],当敲除 ErbB2 阳性乳腺癌细胞系中 ErbB2 基因时,DDX5 蛋白的表达不受影响,反之亦然。因此推测 ErbB2 阳性乳腺癌对 ErbB2 靶向药物如拉帕替尼和曲妥珠单抗的耐药性可能是存在 ErbB2 阳性低/DDX5 高的表达情况,在靶向 ErbB2 的基础上加上 DDX5 的联合治疗对 ErbB2 乳腺癌的治疗可能会更优[10]。更有研究发现 DDX5 在淋巴结侵犯、分化程度差的乳腺癌患者中含量升高[6]。综上,DDX5 可通过多个途径,多靶点促进乳腺癌侵袭和转移,确定 DDX5 调控机制以及其与调控通路上相关蛋白的复杂相互作用对于乳腺癌的精准靶点的选取具有意义。

3　DDX5 在乳腺癌组织中的表达及临床意义

精确诊断以及预后、复发在乳腺癌临床治疗中具有重要意义,尽管随着乳腺癌诊治的深入,其生存率有所提高,但新的生物分子的确定以进一步精确乳腺癌的分型诊断、评判预后和复发对于制定更优效的治疗很有必要,DDX5 在乳腺癌不同分型表达的差异性以及与复发预后的特异性指标的关系预示着其成为新的乳腺癌标志物的可能性。

3.1　DDX5 *与乳腺癌分型*　肿瘤异质性在乳腺癌的诊治中仍为一个巨大挑战,异质性的分子的确定对于不同乳腺癌亚型的发生、发展、治疗、预后至关重要,然而研究发现 DDX5 在不同的乳腺癌亚型中的表达存在差异,是乳腺癌异质性分子标志物之一,DDX5 的平均相对表达从管腔亚型到基底亚型逐渐增加,基底 B 细胞系(包含 TNBC)的平均值最高达 1.17,与 Ki-67 和 EGFR 的表达呈正相关[10]。He[30]的研究结果与之一致,并与之采用相同的 BT549 TNBC 细胞,相比于 luminal A、luminal B 和富含 HER2(即 ErbB2)的亚型,BT549 细胞 DDX5 表达水平最高,免疫荧光定量值接近于 2。上述研究与 Li 等[6]的研究却不一致,研究显示 DDX5 在 luminal B 型乳腺癌表达最丰富,而 Mazurek 等[8]的研究也早已发现 DDX5 在 luminal B 型扩增频率最高,达 53%。上述的差异提示 DDX5 的表达对于乳腺癌异质性的判断是有优势的,重点在于确定对应

相关乳腺癌类型的 DDX5 具体表达含量。有趣的是 Li 等[6]的研究还揭示 DDX5 升高的乳腺癌患者与 ER 阴性/PR 阳性状态相关。然而在区分良恶性乳腺肿瘤上 DDX5 也发挥了作用，DDX5 在 54 例乳腺恶性肿瘤组织中呈现核强染色，阳性率达 79.4%(54/68)，而良性组织阳性率为 0[10]。并且在一项研究中，相比于乳腺肿瘤，DDX5 在 6 种正常乳腺组织中的表达无差异[30]。

3.2　DDX5 与乳腺癌复发与预后　DDX5 是乳腺癌患者重要的独立预后因素，在 Li 等[22]的研究中，DDX5 高表达的乳腺癌患者总生存期(OS)、无病生存期 (DFS)比低表达缩短 4 年左右，临床预后结局较差。Li 等[6]研究确定了 DDX5 是有效的乳腺癌复发以及预后的生物标志物，研究 868 例乳腺癌患者后发现，与周围正常组织相比 DDX5 在乳腺癌患者中高表达，DDX5 高表达的患者复发风险非常高($P<0.01$)，与晚期 TNM 分期、淋巴结侵犯、分化不良显著相关($P<0.01$)，与无复发生存期(RFS)和乳腺癌特异性生存期(BCSS)负相关的同时伴随生存期的缩短。与之一致的是，对 801 名 ER 阴性乳腺癌患者 DDX5 表达水平与患者预后相关性分析可知，DDX5 高表达患者预后差($P=0.002$)，与 RFS 负相关。此外，DDX5 含量较低的 ER 阳性乳腺癌患者，与患者预后不相关($P=0.51$)。接下来对乳腺癌预后基因数据集(GOBO)分析发现无远处转移生存率(DMFS)在 DDX5 调控的增殖促进基因高表达组中降低，而增殖抑制基因集的高表达组增高，预示着良好预后[30]。这彰显了 DDX5 调控的基因网络在预测乳腺癌临床预后的价值。然而 Liu 等[27]发现 DDX5 的 mRNA 表达在伴有肿瘤 T 期进展的乳腺浸润性癌(breast invasive carcinoma, BRCA)中降低，但是 DDX5 蛋白在乳腺癌总表达上增高，更加肯定了 DDX5 在转录过程中复杂调节机制，同样肯定了 DDX5 作为评估肿瘤预后生物标志物的价值。

4　中药干预 DDX5 与乳腺癌

人参皂苷 Rh1(ginsenoside-Rh1, G-Rh1)是传统中草药人参的提取物，能通过线粒体活性氧(oxygen species, ROS)的积累破坏线粒体电位、阻滞 G1/S 期、裂解半胱天冬酶-3 的活化促进 TNBC 的凋亡[39]。张译丹[40]在体外细胞实验中发现 500 μmol/L 的 G-Rh1 能将 HER2 阳性乳腺癌细胞阻滞于 S 期并诱导晚期凋亡，抑制 DDX5 的表达，而 DDX5 在 HER2 阳性乳腺癌细胞的阳性率为 60%(21/35)，随着 DDX5 表达的增加，淋巴结转移增多、Ki-67 水平增高、临床 TNM 分期越晚、病理组织学分级越差。由此推测 G-Rh1 并未通过影响 DDX5 基因表达而是促进 DDX5 转录后的蛋白降解来实现对 HER2 阳性乳腺癌细胞恶性生物学行为的逆转，认为 DDX5 是 HER2 阳性乳腺癌预后标记物和治疗新靶点。G-Rh1 降解 DDX5 蛋白以及干预 DDX5 转录的具体机制值得钻研，鉴于 DDX5 在乳腺癌的表达的丰富性、特异性以及未来靶向治疗可能存在的耐药性的问题，中药联合干预 DDX5 的靶向治疗对于乳腺癌患者可能是一个新方向，因而更多的中药干预 DDX5 蛋白的研究应是后续探索方向。

5　结语与展望

DDX5 发挥的对乳腺癌增殖、侵袭、转移的促进作用为进一步乳腺癌新分子标靶确立及相关干预药物研究提供新见解，同时在预测乳腺癌分子亚型、预后、复发方面发挥着重要作用，对于乳腺癌患者来说是一个不错的分子靶向治疗选择。因此，DDX5 自身以及与相关上下游通路基因作用于乳腺癌的具体机制应深入研究，为进一步针对干预 DDX5 的乳腺癌药物研制以及精准分子标靶提供参考。随着中药抗肿瘤的优势逐渐被挖掘，结合中药调控 DDX5 蛋白干预乳腺癌发展预后的报道，应考虑将中药与 DDX5 靶向治疗结合，为乳腺癌患者谋求一个更好的治疗道路。

利益冲突　无

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