Immunohistochemical expression of NEDD9, E-cadherin and γ-catenin and their prognostic significance in pancreatic ductal adenocarcinoma (PDAC)

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ABSTRACT

Extensive research is being conducted to identify novel diagnostic, predictive and prognostic biomarkers for pancreatic ductal adenocarcinoma (PDAC), as only a few markers have been routinely used so far with limited success. Our aim was to assess the expression of neural precursor cell expressed developmentally down-regulated protein 9 (NEDD9), E-cadherin, and γ-catenin in PDAC in relation to clinicopathological parameters and patient survival. We also investigated if there is a correlation of NEDD9 expression with E-cadherin or γ-catenin. The protein expression was determined by immunohistochemistry in 61 PDAC and 61 samples of normal pancreatic tissue. The log rank test and Kaplan-Meier survival curve were used for survival analysis. E-cadherin and γ-catenin expressions were reduced in PDAC, and completely retained in normal pancreatic tissue. Expression of NEDD9 was significantly increased in PDAC (strong expression in 78.7% of cases and moderate in 21.3%) and reduced in normal pancreatic tissue (strong positivity in 45.9% of cases, moderate in 31.1%, and weak in 23%). There was a positive correlation between reduced E-cadherin and γ-catenin expression in PDAC (p = 0.015). The loss or reduced expression of E-cadherin had a negative impact on patient survival (p = 0.020). A negative correlation between E-cadherin expression and tumor grade was also observed (p = 0.011). Decreased E-cadherin expression was more common in male patients with PDAC (81.3% vs. 60% for females, p = 0.005). γ-catenin and NEDD9 expressions were not statistically correlated with tumor stage and grade, gender, nor with patient survival. Our results support the role of NEDD9, E-cadherin and γ-catenin proteins in PDAC, but further research should clarify in detail their mechanism of action in pancreatic cancer.

KEY WORDS: Pancreatic ductal adenocarcinoma; PDAC; NEDD9; E-cadherin; γ-catenin; immunohistochemistry
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INTRODUCTION

In developed countries, pancreatic cancer (PC) remains in the top 5 of most frequent causes of death due to cancer. Furthermore, the incidence of PC, although still low in most populations compared to other cancers, has been increasing steadily in the past years [1]. The most frequent type of PC is pancreatic ductal adenocarcinoma (PDAC), an aggressive form originating from the epithelium of the pancreatic duct. Despite improvements in PC research, the etiology of PDAC, reliable markers for early diagnosis and effective treatment options remain largely undetermined, resulting in a high mortality rate and short survival time after recognition of the disease. Considering that, up until now, only a few PDAC markers have been routinely used in clinical practice with limited sensitivity and specificity, extensive research is being conducted to identify novel diagnostic, predictive and prognostic biomarkers for PDAC.

Neural precursor cell expressed developmentally down-regulated protein 9 (NEDD9), also known as EF1 and CAS-L, was originally identified based on its downregulated expression pattern during the development of mouse brain [2,3]. NEDD9 is a member of Crk-associated substrate (CAS) family of adaptor molecules which also includes breast cancer anti-estrogen resistance protein 1 (BCAR1 or p130Cas), embryonal fyn-associated substrate (EFS or SIN), and Cas scaffolding protein family member 4 (CASS4 or HEPL). NEDD9 has been implicated in cancer and its overexpression was associated with tumor progression. For example,

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activation of NEDD9 was demonstrated in melanoma where it could enhance cancer cell invasion *in vitro* and metastasis in a mouse model *in vivo* [4]. Moreover, NEDD9 overexpression directed elongation and mesenchymal-type invasion in melanoma cells by engaging integrin avb3 and recruiting Src kinase [5]. A recent study [2] also showed increased mRNA and protein levels of NEDD9 in pancreatic carcinoma lesions compared to the paired adjacent non-tumor tissue. In the same study, a high NEDD9 expression was statistically correlated with clinical staging, lymph node metastasis, and histological differentiation [2].

E-cadherin or cadherin-1 is a type of calcium-dependent transmembrane glycoprotein with a function in mediating cell-cell adhesion between epithelial cells. Structurally, E-cadherins are composed of three domains: an extracellular domain, which extends from the surface of the cell and binds to cadherins on adjacent cells, transmembrane domain composed of glycoprotein repeats, and intracellular or cytoplasmic domain which binds various cytoplasmic proteins, including catenins. There are four types of catenins: α, β, δ and γ. E-cadherin specifically binds to β- or γ-catenin, while α-catenin binds either to β-catenin or γ-catenin, but not to E-cadherin. δ- or p120-catenin binds to a juxtamembrane domain of E-cadherin. β-catenin possesses an armadillo domain which consists of characteristic repeats folded together into an elongated shape and functions as a ligand-binding site. β-catenin is also the main component of the Wnt signaling pathway and can act as an oncogene [6]. α-catenin links the cadherin-catenin complex to actin filaments via vinculin and alpha actins. α-catenin can also regulate the arrangement of actin filaments [7,8]. γ-catenin or plakoglobin is a component of desmosomes but may also bind to classical cadherins. It has been implicated in the control of epithelial cell motility, i.e. low levels of γ-catenin correlated with higher cell motility [9,10]. Overall, these cell-cell contacts maintain the structural integrity of tissues [7,8], and lower E-cadherin expression or complete loss have been linked to several tumor types.

The aim of this study was to evaluate immunohistochemical expression of NEDD9, E-cadherin and γ-catenin in PDAC patients in relation to clinicopathological parameters and patient survival. We also investigated if there is a correlation of NEDD9 expression with E-cadherin or γ-catenin in PDAC, as E-cadherin was demonstrated to be a downstream target of NEDD9.

MATERIALS AND METHODS

Samples and patients

We obtained 61 tumor and 61 normal pancreatic tissue samples from patients with PDAC who underwent pancreatectomy at our institution between 2000 and 2012. All slides were re-evaluated for the purpose of this study. There were 30 male and 31 female patients, with the age range 32–78 years.

Immunohistochemistry

The expression of NEDD9, E-cadherin and γ-catenin was determined in 61 samples of PDAC and 61 normal pancreatic tissues by measuring the intensity of immunohistochemical staining and percentage of positive tumor cells. Immunohistochemical analyses were performed using formalin-fixed, paraffin-embedded (FFPE) tissue sections (thickness 5 μm). Deparaffinization and immunohistochemical staining were carried out using a microwave streptavidin immunoperoxidase (MSIP) protocol and labeled streptavidin-biotin (LSAB) method on a DAKO TechMate™ Horizon automated immunostainer (DAKO, Denmark). Monoclonal antibodies were directed against NEDD9 (sab 4200376, dilution 1:200, R Sigma, USA), E-cadherin (Clone NCH-38, dilution 1:50, Dako, Denmark) and γ-catenin (ab 15153, dilution 1:100, R Sigma, USA).

Immunoreaction was detected in epithelial tumor cells as well as in the epithelial component of metastatic lymph nodes in the areas with the highest activity ("hot spots") under magnification of ×400, for a total of 1,000 tumor cells. The "hot spot" was determined upon inspection of the whole section (magnification of ×40).

The immunohistochemical staining for NEDD9 and γ-catenin was scored as follows: 0 - no reaction or weak cytoplasmic staining in <25% of tumor cells; 1 - weak cytoplasmic staining in >25% of tumor cells; 2 - moderate staining in >25% of tumor cells; and 3 - strong staining in >25% of tumor cells [4].

E-cadherin staining was considered absent when the staining was either absent or present only in <5% of cancer cells. The staining was considered positive if the intensity was strong (2+) or weak (1+) and if it was detected in ≥5% of tumor cells. As previously described, we classified E-cadherin expression as "intact" when 100% of tumor cells in the tissue were stained, "focal loss" when ≥51% and <99% of tumor cells were labeled, "diffuse loss" when ≥6% and ≤50% of tumor cells were labeled and "total loss" when <5% of tumor cells were labeled. We did not evaluate other cut-offs of expression percentages [11].

Follow-up data

Additional information was collected as follows: patient age, gender, tumor size, tumor grade, number of resected lymph nodes, number of positive lymph nodes, follow-up duration, and survival time.

Statistical analysis

Nominal categorical data were presented as frequencies and proportions, while numerical data were shown as medians.

The normality of distribution was tested by the Kolmogorov–Smirnov test. NEDD-9, γ-catenin and E-cadherin expression values were compared between tumor and control samples by chi-squared test. A relationship between clinicopathological parameters and immunohistochemical markers was analyzed by Spearman's rank correlation coefficient. The log rank test and Kaplan-Meier survival curve were used to analyze survival in relation to immunohistochemical markers. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY).

RESULTS

Clinicopathological characteristics of patients with PDAC

The study included 30 male and 31 female patients with the age ranging from 32 to 78 years (median 62). Tumor size ranged from 1.5 to 10 cm (median 3.5), and the majority of tumors (59%) were grade 2. Lymph nodes were negative for tumor cells in 26 and positive in 29 cases. In 6 cases, lymph nodes were not sampled. In 3 cases, metastases were present at the time of operation. The number of positive lymph nodes in each patient varied from 0 to 6 with the mean proportion of positive lymph nodes being 20.95%. The follow-up ranged 1–139 months (median 12.4). In that period, 52 patients died of the disease, 7 were still alive, and for 2 patients there was no data about survival.

Immunohistochemical expression of NEDD9, γ-catenin, and E-cadherin

In PDAC group, the expression of NEDD9 was strong in 78.7% cases, moderate in 21.3%, and there was no case with weak expression of NEDD9. In control group, normal pancreatic tissue was strongly positive for NEDD9 in 45.9% of cases, moderately positive in 31.1%, and weakly positive in 23% of cases. The difference in NEDD9 expression was significantly different between the two groups.

E-cadherin expression was analyzed as the intensity of cytoplasmic staining as well as preserved and focally or diffusely lost expression. We found that E-cadherin expression was completely intact and strongly positive in all cases of normal pancreatic tissue, while in PDAC tissue E-cadherin expression was mostly weak (73.8% of cases) and focally lost (45.9 % of cases). In PDAC group, low E-cadherin expression was more common in male (60%, $p < 0.005$) and higher E-cadherin expression in female patients (81.3%). Moreover, weak staining of E-cadherin was more often found in moderate- (62.2%) and high-grade PDAC (26.7%), strong E-cadherin staining was more common in moderate- (50%) and low-grade PDAC [43.8%] (Table 1, Figure 1). E-cadherin expression was statistically more often ($p < 0.001$) diffusely (24.4%) or focally (57.8%) lost in PDAC samples with weak staining, while in specimens with strong E-cadherin staining the expression was preserved in the majority of cases (81.3%). In normal pancreatic tissue, γ-catenin expression was high in

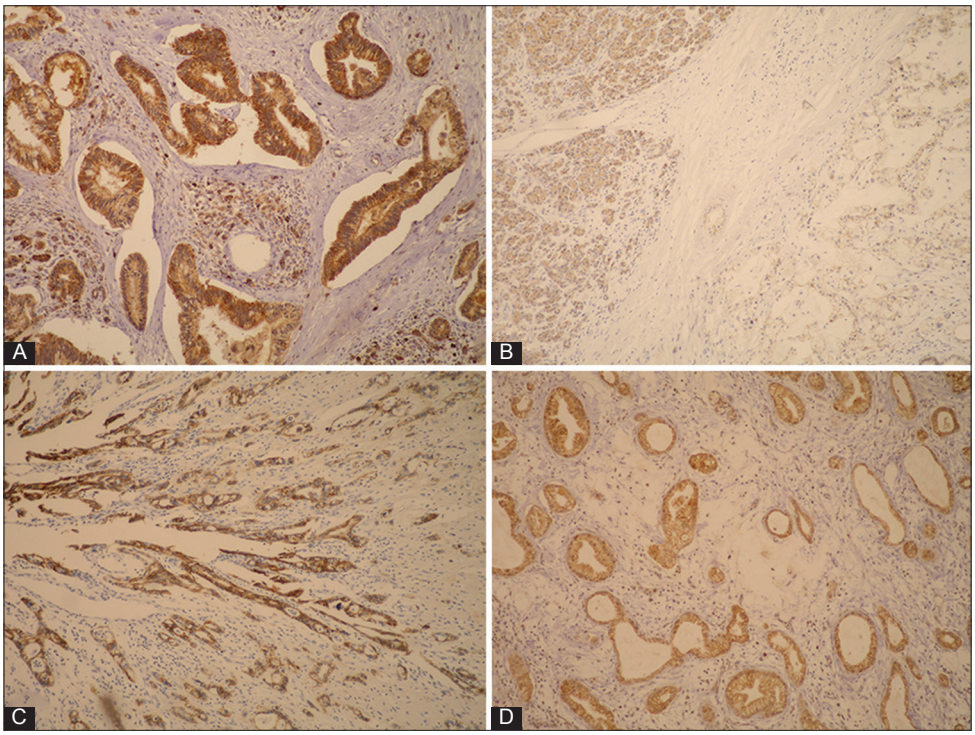

FIGURE 1. (A) High expression of neural precursor cell expressed developmentally down-regulated protein 9 (NEDD9) in pancreatic ductal adenocarcinoma [PDAC] (200×). (B) High expression of E-cadherin in normal pancreatic tissue [left] and low expression in PDAC [right] (100×). (C) High expression of E-cadherin in PDAC (100×). (D) High γ-catenin expression in PDAC (100×).

TABLE 1. Immunohistochemical expression of E-cadherin, neural precursor cell expressed developmentally down-regulated protein 9 (NEDD-9), and γ-catenin in relation to clinicopathological data of patients with pancreatic ductal adenocarcinoma (PDAC)

Immunohistochemical expression in tumor		E-cadherin				NEDD9						γ-catenin					
		Low (1)		High (2)		Low (1)		Medium (2)		High (3)		Low (1)		Medium (2)		High (3)	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Gender	Male	27	60	3	18.8	0	0	6	46.2	24	50	1	100	10	62.5	19	43.2
	Female	18	40	13	81.3	0	0	7	53.8	24	50	0	0	6	37.5	25	56.8
Tumor	1	4	8.9	2	12.5	0	0	1	7.7	5	10.4	0	0	2	12.5	4	9.1
	2	8	17.8	3	18.8	0	0	4	30.8	7	14.6	0	0	3	18.8	8	18.2
	3	32	71.1	11	68.8	0	0	8	61.5	35	72.9	1	100	11	68.8	31	70.5
	4	1	2.2	0	0	0	0	0	0	1	2.1	0	0	0	0	1	2.3
Node	0	18	45	7	46.7	0	0	4	36.4	21	47.7	1	100	5	35.7	19	47.5
	1	22	55	8	53.3	0	0	7	63.6	23	52.3	0	0	9	64.3	21	52.5
Metastasis	1	3	100	0	0	0	0	0	0	3	100	0	0	2	100	1	100
Grade	1	5	11.1	7	43.8	0	0	1	7.7	11	22.9	0	0	2	12.5	10	22.7
	2	28	62.2	8	50	0	0	9	69.2	27	56.3	1	100	11	68.8	24	54.5
	3		12	26.7	1	6.3	0	0	3	23.1	10	20.8	0	0	3	18.8	10

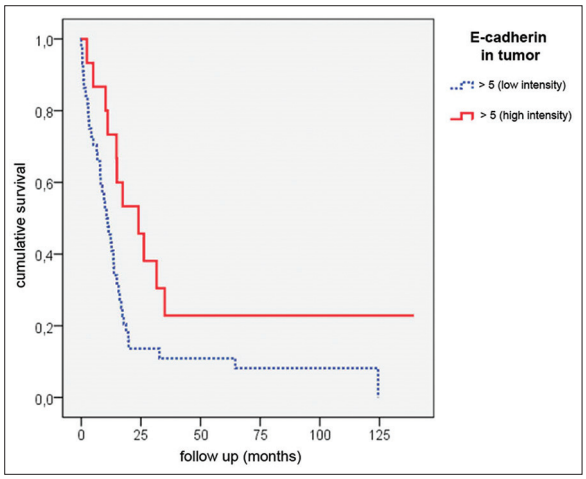

FIGURE 2. The Kaplan-Meier analysis of cumulative survival in relation to E-cadherin expression in pancreatic ductal adenocarcinoma [PDAC]. A positive correlation between E-cadherin expression and patient survival was observed (log rank test; $p = 0.020$); i.e. patients with higher E-cadherin expression had longer survival after pancreatectomy.

98.4% and moderate in 16% of cases. In PDAC tissue, γ-catenin expression was high in 72.1%, moderate in 26.2%, and low in 1.6% of specimens (Figure 1).

Survival analysis

We observed a positive correlation between reduced E-cadherin and γ-catenin expression in PDAC group, as well as a positive correlation between E-cadherin expression and patient survival (log rank test, $p = 0.020$); i.e. patients with higher E-cadherin expression had longer survival after pancreatectomy (Figure 2). γ-catenin expression was not statistically correlated with the tumor stage, tumor grade, gender, nor with patient survival. Also, there was no correlation between NEED9 and survival time, despite higher NEED9 expression in tumor samples.

DISCUSSION

To be able to spread beyond the primary tumor site cancer cells must undergo changes in molecular mechanisms that control cell proliferation, growth, ability to avoid immune response, and cell-cell interaction. Changes in the expression of NEDD9 have been associated with hyperproliferation and invasion in some tumors. As a scaffold protein, NEDD9 assembles signaling complexes with other molecules, which then regulate multiple cellular processes, most of them being important for cancer development and progression. Furthermore, by regulating downstream targets, including E-cadherin (and possibly γ-catenin), NEDD9 is able to affect various cellular functions and processes such as migration, invasion, survival and multiplication. Although NEDD9 most probably does not have a direct oncogenic effect, a higher expression of NEDD9 is commonly observed in tumor cells and affects tumor aggressiveness and response to treatment [12].

A number of studies showed that NEDD9 overexpression is associated with metastasis in different cancer types, including breast cancer, glioblastoma, melanoma and cervical cancer [4,13-15]. Štajduhar et al. found increased expression of NEDD9 in epithelial and stromal components of axillary lymph node metastases of breast cancer compared to non-metastatic tumors [13]. Another study showed that NEDD9 could enhance metastasis in an inducible mouse model of melanoma [4]. Moreover, NEDD9 together with BCAR1 promoted the removal of E-cadherin from the cell membrane and lysosomal degradation through Src kinase in MCF7 breast adenocarcinoma cells, thus probably contributing to the epithelial-mesenchymal transition (EMT) of tumor cells [16]. Nevertheless, in our study, there was no correlation between NEDD9 and E-cadherin expression in PDAC cells.

Other studies showed that higher expression of NEDD9 was characteristic for aggressive tumors positive for Ras activation, p16 inhibition, *BCR-ABL* translocation, or transformation of human T-cell lymphotropic virus type 1 (HTLV-1)-infected cells [4,17]. These initial molecular changes, associated with increased NEDD9 expression, lead to the inhibition of apoptosis or inactivation of the mitotic checkpoint, and thus promote the invasive potential of tumor cells. In their review, Mahendra et al. indicated the important role of NEDD9 in the regulation of cell cycle, apoptosis, cell attachment, migration, and invasion [18]. Xue et al. [2] showed higher mRNA and protein NEDD9 levels in 106 PDAC compared to adjacent normal tissue. Higher expression of NEDD9 was correlated with higher tumor stage, lower differentiation and with lymph node metastases. Moreover, they reported a negative correlation between patient survival and NEDD9 expression and indicated that NEDD9 might serve as an independent factor of poor prognosis [2]. Our study also showed significantly higher expression of NEDD9 in PDAC (Figure 1) compared to normal pancreatic tissue, however, we did not observe a correlation between NEDD9 and tumor grade, stage or patient survival.

E-cadherin expression was lower in our PDAC group compared to normal pancreatic tissue (Figure 1). Different mechanisms may lead to E-cadherin inactivation in malignant cells, including mutations, DNA methylation, gene silencing and endocytosis. Endocytosis and degradation of E-cadherin can be the result of the activation of proto-oncogenes such as Src and epidermal growth factor receptor (EGFR), as shown in a study by Nagathihalli and Mershant [19] where higher activity of Src tyrosine kinase was common in PDAC, regulating E-cadherin function and EMT and leading to increased tumor progression, invasion and metastasis [19]. In our study, lower E-cadherin expression was more common in higher-grade tumors, confirming the role of E-cadherin in tumor progression. Pećina-Šlaus et al. found lower E-cadherin expression in higher-grade meningeomas [20]. Hong et al. [11] also demonstrated that lower expression of E-cadherin was more common in high-grade PDAC, while Pryczynicz et al. [21] did not find a correlation between E-cadherin expression and tumor grade or lymph node metastases. An *in vitro* study using JHP-1 adenocarcinoma cell line showed that the downregulation of E-cadherin in pancreatic carcinoma cells promoted the invasiveness into the basement membrane, and similarly *in vivo* selection of highly metastatic murine pancreatic cancer cells induced EMT by genetic inactivation of E-cadherin in parental cells [22,23].

Focally lost E-cadherin expression was more common in our sample and it was also correlated with intermediate γ-catenin expression. Lupu-Meiri et al. suggested plasminogen activator inhibitor-1 (PAI-1) as another factor that suppresses differentiation of PDAC cells by inhibiting E-cadherin expression [24].

Other studies confirmed that total or partial loss of E-cadherin expression is correlated with shorter survival of patients [11,25]. In the present study, patient survival was positively correlated with preserved and high E-cadherin expression, which may be explained by higher cell adhesions in those PDACs that were less likely to migrate and metastasize. Therefore, our study confirms that diminished expression of E-cadherin strongly affects EMT and metastatic potential of tumor cells.

We observed lower E-cadherin expression more frequently in male patients and higher in female patients, but there was no significant difference in survival between men and women. Contrary to our study, Pryczynicz et al. did not find a correlation between E-cadherin expression and patient gender [21].

We detected lower γ-catenin expression in PDAC compared to normal pancreatic tissue, where its expression was high. γ-catenin has an important role in maintaining epithelial integrity and despite having the same binding site to E-cadherin as β-catenin, it has a different oncogenic activity. Generally, a tumor-suppressor role has been suggested for γ-catenin [21,26]. Charpentier et al. [27] found that even modest expression of two γ-catenin transgenes was able to decrease the proliferation in basal epidermal cells in mouse and to significantly reduce the length of the growth phase in the hair follicle cells [27]. Also, studies showed that, in a cell culture, keratinocytes lacking γ-catenin were less adherent and exhibited a higher cell migration [3]. In addition, lower γ -catenin expression was correlated with the invasiveness of breast cancer [28]. Nevertheless, our results indicated no correlation between γ-catenin expression and patient survival.

CONCLUSION

In summary, reduced and focally or diffusely lost expression of E-cadherin and reduced expression of γ-catenin was observed in PDAC, while high and completely preserved E-cadherin expression and high γ-catenin expression was detected in normal pancreatic tissue. Our study indicated a strong correlation of reduced E-cadherin expression with reduced γ-catenin expression, tumor grade, and patient survival. On the other hand, expression of NEDD9 was increased in PDAC and reduced in normal pancreatic tissue. Overall, our results support the role of NEDD9 and cadherin/catenin proteins as biomarkers in PDAC, as well as their use in developing more effective therapies for this aggressive form of cancer. Nevertheless, additional studies are necessary to clarify in detail the mechanism of action and interaction of these proteins in PDAC.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

[1] Raimondi S, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: An overview. Nat Rev Gastroenterol Hepatol 2009;6(12):699-708. https://doi.org/10.1038/nrgastro.2009.177.

[2] Xue YZ, Sheng YY, Liu ZL, Wei ZQ, Cao HY, Wu YM, et al. Expression of NEDD9 in pancreatic ductal adenocarcinoma and its clinical significance. Tumour Biol 2013;34(2):895-9. https://doi.org/10.1007/s13277-012-0624-8.

[3] Law SF, Estojak J, Wang B, Mysliwiec T, Kruh G, Golemis EA. Human enhancer of filamentation 1, a novel p130cas-like docking protein, associates with focal adhesion kinase and induces pseudohyphal growth in Saccharomyces cerevisiae. Mol Cell Biol 1996;16(7):3327-37. https://doi.org/10.1128/MCB.16.7.3327.

[4] Kim M, Gans JD, Nogueira C, Wang A, Paik JH, Feng B, et al. Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene. Cell 2006;125(7):1269-81. https://doi.org/10.1016/j.cell.2006.06.008.

[5] Ahn J, Sanz-Moreno V, Marshall CJ. Metastasis gene NEDD9 acts through integrin b3 and Src to promote mesenchymal motility and inhibit amoeboid motility. J Cell Sci 2012;125(7):1814-26. https://doi.org/10.1242/jcs.101444.

[6] Weis WI, Nelson WJ. Re-solving the cadherin-catenin-actin conundrum. J Biol Chem 2006;281(47):35593-7. https://doi.org/10.1074/jbc.R600027200.

[7] Drees F, Pokutta S, Yamada S, Nelson WJ, Weis WI. Alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly. Cell 2005;123(5):903-15. https://doi.org/10.1016/j.cell.2005.09.021.

[8] Knudsen KA, Soler AP, Johnson KR, Wheelock MJ. Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. J Cell Biol 1995;130(1):67-77. https://doi.org/10.1083/jcb.130.1.67.

[9] Yin T, Getsios S, Caldelari R, Kowalczyk AP, Muller EJ, Jones JCR, et al. Plakoglobin suppresses keratinocyte motility through both cell-cell adhesion-dependent and -independent mechanisms. Proc Natl Acad Sci U S A 2005;102(15):5420-5. https://doi.org/10.1073/pnas.0501676102.

[10] Todorovic V, Desai BV, Patterson MJ, Amargo EV, Dubash AD, Yin T, et al. Plakoglobin regulates cell motility through Rho- and fibronectin-dependent Src signaling. J Cell Sci 2010;123(20):3576-86. https://doi.org/10.1242/jcs.070391.

[11] Hong SM, Li A, Olino K, Wolfgang CL, Herman JM, Schulick RD, et al. Loss of E-cadherin expression and outcome among patients with resectable pancreatic adenocarcinoma. Mod Pathol 2011;24(9):1237-47. https://doi.org/10.1038/modpathol.2011.74.

[12] Shagisultanova E, Gaponova AV, Gabbasov R, Nicolas E, Golemis EA. Preclinical and clinical studies of the NEDD9 scaffold protein in cancer and other diseases. Gene 2015;567(1):1-11. https://doi.org/10.1016/j.gene.2015.04.086.

[13] Štajduhar E, Sedić M, Leniček T, Radulović P, Kerenji A, Krušlin B, et al. Expression of growth hormone receptor, plakoglobin and NEDD9 protein in association with tumour progression and metastasis in human breast cancer. Tumour Biol 2014;35(7):6425-34. https://doi.org/10.1007/s13277-014-1827-y.

[14] Natarajan M, Stewart JE, Golemis EA, Pugacheva EN, Alexandropoulos K, Cox BD, et al. HEF1 is necessary and specific downstream effector of FAK that promotes the migration of glioblastoma cells. Oncogene 2006;25(12):1721-32. https://doi.org/10.1038/sj.onc.1209199.

[15] Sima N, Cheng X, Ye F, Ma D, Xie X, Lü W. The overexpression of scaffolding protein NEDD9 promotes migration and invasion in cervical cancer via thyrosine phosphorylated FAK and SRC. PlosOne 2013;8(9):e74594. https://doi.org/10.1371/journal.pone.0074594.

[16] Tikhmyanova N, Golemis EA. NEDD9 and BCAR1 negatively regulate E-cadherin membrane localization, and promote E-cadherin degradation. PLoS One 2011;6(7):e22102. https://doi.org/10.1371/journal.pone.0022102.

[17] Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, et al. Genes that mediate breast cancer metastasis to lung. Nature 2005;436(7050):518-24. https://doi.org/10.1038/nature03799.

[18] Singh M, Cowell L, Seo S, O'Neill G, Golemis E. Molecular basis for HEF1/NEDD9/Cas-L action as a multifunctional coordinator of invasion, apoptosis and cell cycle. Cell Biochem Biophys 2007;48(1):54-72. https://doi.org/10.1007/s12013-007-0036-3.

[19] Nagathihalli NS, Mershant NB. Src-mediated regulation of E-cadherin and EMT in pancreatic cancer. Front Biosci (Landmark Ed) 2012;17:2059-69. https://doi.org/10.2741/4037.

[20] Pećina-Šlaus N, Nikuseva Martić T, Deak AJ, Zeljko M, Hrasćan R, Tomas D, et al. Genetic and protein changes of E-cadherin in meningiomas. J Cancer Res Clin Oncol 2010;136(5):695-702. https://doi.org/10.1007/s00432-009-0708-z.

[21] Pryczynicz A, Guzińska-Ustymowicz K, Kemona A, Czyzewska J. Expression of the E-cadherin-catenin complex in patients with pancreatic ductal adenocarcinoma. Folia Histochem Cytobiol 2010;48(1):128-33. https://doi.org/10.2478/v10042-008-0089-1.

[22] Takao S, Che X, Fukudome T, Natsugoe S, Ozawa M, Aikou T. Downregulation of E-cadherin by antisense oligonucleotide enhances basement membrane invasion of pancreatic carcinoma cells. Hum Cell 2000;13(1):15-20.

[23] Von Burstin J, Eser S, Paul MC, Brandl M, Messer M, von Werder A, et al. E-cadherin regulates metastasis of pancreatic cancer in vivo and is suppressed by SNAIL/HDAC1/HDAC2 repressor complex. Gastroenterology 2009;137(1):361-71. https://doi.org/10.1053/j.gastro.2009.04.004.

[24] Lupu-Meiri M, Geras-Raaka E, Lupu R, Shapira H, Sandbank J, Segal L, et al. Knock-down of plasminogen-activator inhibitor-1 enhances expression of E-cadherin and promotes epithelial differentiation of human pancreatic adenocarcinoma cells. J Cell Physiol 2012;227(11)3621-8. https://doi.org/10.1002/jcp.24068.

[25] Hsu HP, Shan YS, Jin YT, Lai MD, Lin PW. Loss of E-cadherin and beta-catenin is correlated with poor prognosis of ampullary neoplasms. J Surg Oncol 2010;101(5):356-62. https://doi.org/10.1002/jso.21493.

[26] Toyoda E, Doi R, Koizumi M, Kami K, Ito D, Mori T, et al. Analysis of E-, N-cadherin, alpha-, beta-, and gamma-catenin expression in human pancreatic carcinoma cell lines. Pancreas 2005;30(2):168-73. https://doi.org/10.1097/01.mpa.0000148514.69873.85.

[27] Charpentier E, Lavker RM, Acquista E, Cowin P. Plakoglobin suppresses epithelial proliferation and hair growth in vivo. J Cell Biol 2000;149(2):503-20. https://doi.org/10.1083/jcb.149.2.503.

[28] Kolligs FT, Kolligs B, Hajra KM, Hu G, Tani M, Cho KR, et al. Gamma-catenin is regulated by the APC tumor suppressor and its oncogenic activity is distinct from that of beta-catenin. Genes Dev 2000;14(11):1319-31. https://doi.org/10.1101/gad.14.11.1319.