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δ -Catenin as potential cancer biomarker

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To the Editors

δ -Catenin/NPRAP/Neurojungin (gene designation: *CTNND2*) is primarily a neuronal postsynaptic protein expressed in the brains of healthy individuals.¹⁻³ However, it is increasingly recognized that this initially described neural specific member of the β -catenin/armadillo superfamily is overexpressed in cancers of peripheral tissues, including prostate, breast, lung, and ovarian cancers.⁴⁻⁶ Our previous studies demonstrated that δ -catenin mRNA and protein are overexpressed in primary prostatic adenocarcinomas.^{4,7} Since then, a number of excellent studies have provided strong evidence that δ -catenin overexpression is associated with poor prognosis in cancer, including lung cancer.⁵ These studies significantly elevated the expectation that δ -catenin overexpression is a potential cancer biomarker.

From the studies of δ -catenin expression and distribution in different cancer types, it is clear that δ -catenin could be expressed as different variants with modifications and localized to different subcellular regions. Increased pathology literature on δ -catenin involvement in cancer progression calls for careful interpretation of δ -catenin expression data to avoid potential misunderstanding of its functions in carcinogenesis. When cancer tissues are examined, it is important to determine whether the full-length, modified fragments, or different variants of δ -catenin are expressed in the cancer cells and whether the revealed δ -catenin distribution in cancer is dependent upon the choices of antibody applications.

In this letter, we would like to emphasize and raise awareness of epitope dependent δ -catenin expression in different types of cancer cells. When rAb62, a well-characterized antibody against the epitope N-terminal to the armadillo domain,³ was used for Western blot analysis of human cancer cells in culture, it reacted with the predicted δ -catenin full-length protein in TF-1 (*erythroleukemia*), SK-OV-3 (*ovarian adenocarcinoma*), LNCaP (*lymphatic metastasis of prostate cancer*), and MCF7 (*mammary gland/breast adenocarcinoma*), but not NCI-H1299 (*lymph node metastasis of non-small cell lung cancer*) cells (Figure 1A, upper panel). The same pattern was observed for many other cancer cells, including Panc-1 (*pancreas/duct epithelioid carcinoma*), Jurkat cells (*acute T cell leukemia*), CWR22-Rv1 (derived from *prostate tumor xenografts*), PC-3 (*prostate cancer bone metastasis*), DU145 (*brain metastasis of prostate tumor*), HT-29 (*Colorectal Adenocarcinoma*) and more (data not shown). It is of interest to note that a modified and slow migrating high molecular weight form of δ -catenin was detected in TF-1, SK-OV-3

and MCF7 cells (Figure 1A, upper panel). In addition, a number of fragments can be seen in most cancer cells. However, neither the δ -catenin full-length nor the slow migrating modification of δ -catenin was a major protein species in NCI-H1299 lung cancer cells (Figure 1A, upper panel). PZ-HPV-7, an epithelial cell line derived from normal prostate, also showed reduced full-length δ -catenin expression. When we examined the same cell lines with rAb25, an antibody against the carboxyl terminus of δ -catenin,⁴ only SK-OV-3 and MCF7 cells showed the predicted δ -catenin full-length protein species (Figure 1A, lower panel). Instead, most cell lines showed a prominent protein band migrating faster than the predicted full-length δ -catenin. The rAb25 antibody reacted with the slow migrating and modified high molecular weight form of δ -catenin, although it may or may not overlap with the forms that reacted with rAb62 (Figure 1A). These data suggest that besides the full-length δ -catenin, there exist other δ -catenin species with either N-terminal or C-terminal truncations.

We then examined 10 cases of esophageal cancer tissue specimens by real-time PCR and RT-PCR using 8 primer sets that span the entire coding region of *δ -catenin* mRNA. For each primer set-initiated PCR, *δ -catenin* mRNA expression was increased in cancer versus benign specimens (Figure 1B). This result established that a full-length *δ -catenin* transcript is overexpressed in esophageal cancer. This data is consistent with our previous studies that showed the expression of full-length δ -catenin in primary prostatic adenocarcinomas.⁴ Real-time PCR confirmed that *δ -catenin* mRNA expression was increased in esophageal cancer specimens when compared to benign specimens (Figure 1C).

We also examined the immunohistochemical staining pattern of δ -catenin expression and distribution in esophageal tissues. rAb62 reacted weakly with the cell-cell junction in the benign glandular epithelial cells (Figure 2A, arrow). In the esophageal adenocarcinoma, δ -catenin recognized by rAb62 was mainly in the cytoplasm with some nuclear staining (Figure 2B, double arrows). Interestingly, rAb25 gave considerably stronger staining intensity than rAb62 did on cell-cell junction in the benign glandular epithelial cells (Figure 2C, arrowhead). In cancerous tissues, rAb25 detected a mixture of cell-cell contact (Figure 2C, arrowhead) and nuclear expression (Figure 2C, double arrowheads). These data suggest that the δ -catenin protein containing the N-terminus is minimally expressed whereas the δ -catenin form containing the carboxyl terminus is abundantly expressed in the benign cells. In the cancerous cells, the distinct staining pattern suggests that the N-terminus of δ -catenin is prominently associated with the cytoplasmic distribution, whereas the carboxyl terminus of δ -catenin could be subject to significant translocation to the nuclei. These studies provide more insight into δ -catenin expression and distribution in cancer cells.

To determine whether the observations of different forms, variants, and degradation fragments of δ -catenin expression in the tissues can be replicated using body fluid, we prepared human urine samples as well as bronchoalveolar lavage (BAL). When human urine samples were examined for δ -catenin expression, both the full-length and fragments were observed in prostate cancer and benign specimens (Figure S1A). The increased expression of δ -catenin in prostate cancer patient urine was consistent with our recent report.⁸ Similarly, when human BAL was examined, both the full-length and fragments were observed in lung cancer and benign specimens (Figure S1B). Therefore, these proof-of-

concept studies provide a basis to develop immunoassays in the future to evaluate the increased δ -catenin expression as a potential cancer biomarker.

δ -Catenin gene locus (5p15.2) is highly susceptible for generating single nucleotide polymorphism (SNP). Compelling recent evidence has linked *CTNND2* SNP or mutations to cancer, myopia, cortical cataract, and Alzheimer's disease.^{7, 9, 10} The examination of Sanger gene mutation database as well as our own unpublished studies demonstrate the existence of functional mutations that could remove *δ -catenin* sequences in the carboxyl terminus. In corroboration with our observations of different variants and fragments and differential distribution of δ -catenin in the cancer cells and tissues reported in this article, it is important for future studies on δ -catenin to recognize that δ -catenin could exist as different forms in cancer tissues and they may exert different roles in cancer progression. The investigations of δ -catenin involved in human disease pathogenesis have accelerated in recent years and strongly support that δ -catenin is a potential cancer biomarker.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Overexpression of δ -catenin in cancer cells and tissues

A. Western blots showing the expression of different forms of δ -catenin in different cancer cells. Upper panel: Western blot using rAb62, an affinity purified antibody against the sequence N-terminal to the armadillo domains. Lower panel: Western blot using rAb25, an affinity purified antibody against the carboxyl-terminus of δ -catenin. MCFi: Triton X-100 insoluble forms of MCF cells; MCFs: Triton X-100 soluble forms of MCF cells. PC12-tet: PC12 cells stably transfected with full length δ -catenin. Molecular weight markers are on the left. **B.** Human esophageal cancer tissue and the adjacent benign tissue were subject to RT-PCR using primers to δ -catenin mRNAs. Here it shows that in multiple cases, using 8 primer sets that cover the entire coding region of δ -catenin, cancer tissues always showed increased δ -catenin mRNA expression. Note. In each pair, the left lane was cancer tissue while the right lane was benign tissue. This result demonstrated that the entire mRNA for δ -catenin coding region is expressed and that the expression is increased in esophageal cancer. **C.** Human esophageal cancer tissue and the adjacent benign tissue were subject to real-time PCR using primers to δ -catenin (*CTNND2*) mRNAs. Like those in the RT-PCR experiment, cancer tissues showed increased δ -catenin (*CTNND2*) mRNA expression.

Figure 2. Differential distribution of δ -catenin in cancer tissues

A and B. rAb62 had weak staining of cell-cell junction (**A**, arrow) in benign tissue but stained intensely the cytoplasm and some nuclei of esophageal adenocarcinomas (**B**, double arrows). **C and D.** rAb25 had strong staining of cell-cell junction (**C**, arrowhead) of benign tissue and also stained intensely the nuclei (double arrowheads) and cell-cell junctions (arrowhead) in esophageal adenocarcinomas. 400x.