N-Cadherin: A Marker Of Epithelial To Mesenchymal Transition In Tumor Progression
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Citation

Abstract
The cadherins comprise a family of Ca2+-dependant adhesion molecules that function to mediate the cell-to-cell binding that is critical to the maintenance of tissue structure and morphogenesis. The epithelial-mesenchymal transition or transformation (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal cells. The term cadherin switching usually refers to a switch from expression of E-cadherin to expression of N-cadherin. Acquisition of mesenchymal markers in carcinoma cells, such as vimentin, N-cadherin, and fibronectin, with the concomitant loss of epithelial E-cadherin, a major constituent of the adherens junctions, in the processes of EMT. Cancer cells derived from epithelium like oral cancer, gastrointestinal cancer, Prostate cancer, breast cancer, Lung cancer and urinary system cancer, inappropriately express N-cadherin and the up regulation of N-cadherin expression has been shown to promote motility and invasion.

INTRODUCTION
N-cadherin was first identified in 1982 (Grunwald et al., 1982) as a 130 kD molecule in the chick neural retina that was protected by calcium from proteolysis, and in 1984 A-CAM was identified (now called N-cadherin) as a molecule that was localised at the adherens junctions (Volk and Geiger, 1984). In the nomenclature of CD antigens the new designation for N-cadherin is CD325. N-cadherin is also known as neural-cadherin, non-epithelial cadherin or cadherin-2. The N-cadherin gene in mice was located on chromosome 18 (Miyatani et al.1989) and via Yeast Artificial Chromosome (YAC) analysis the structure of the human N-cadherin gene was determined, the entire N-cadherin gene was mapped to a 250-kb region on chromosome 18q11.2. The gene is composed of 16 exons, and homology was found not only between human and mouse, but also between N-cadherin and other cadherins (Wallis et al., 1994). N-cadherin typically forms homotypic homophilic interactions; also heterotypic homophilic and heterophilic interactions have been described.1

The epithelial-mesenchymal transition or transformation (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal cells. There are three general subtypes in EMT based simply on the context under which they occur.Type 1 EMT involves primitive epithelial cells transitioning to motile mesenchymal cells as part of gastrulation and primitive neuroepithelial cells generating migrating neural crest cells. Type 2 EMT involves secondary epithelial or endothelial cells transitioning to resident tissue fibroblasts. In mature tissues, these fibroblasts are induced in response to persistent inflammation. Type 3 EMT involves epithelial carcinoma cells in primary nodules transitioning to metastatic tumor cells in order to migrate through the bloodstream.2

The term cadherin switching usually refers to a switch from expression of E-cadherin to expression of N-cadherin, but also includes situations in which E-cadherin expression levels do not change significantly but the cells turn on (or increase) expression of N-cadherin. It also includes examples in which other cadherins replace or are co-expressed with E-cadherin, including R-cadherin, cadherin 11, T-cadherin and even P-cadherin, and the expression of the ‘inappropriate cadherin’ might alter the behavior of the tumor cells.3

The loss of cell to cell adhesion triggered by activation of an EMT program, together with subsequent cell-ECM adhesions mediated by integrins, like β4, β5, and β6, is among the first critical steps of cancer metastasis, invasion, and progression. Acquisition of mesenchymal
markers in carcinoma cells, such as vimentin, N-cadherin, and fibronectin, with the concomitant loss of epithelial E-cadherin, a major constituent of the adherens junctions, in the processes of EMT. Consequently, E-cadherin is considered to be a “master regulator” of EMT. Furthermore, the connection between loss of E-cadherin expression in cancer cells and poor patient prognosis, including increased tumor grade, metastasis, and mortality, has been established by several studies.  

Several studies from current literature have shown de novo expression, re-expression, up-regulation and down-regulation of N-Cadherin in human tumors and tumoral cell lines. We focus here on the pattern of N-cadherin expression in different malignant tumors of the epithelial origin to evaluate its critical contribution to EMT.

**N-cadherin expression in oral cancer**

Shahidul Islam et al (1996), were observed the squamous cell carcinoma derived cell line which is expressed N-cadherin and displayed a scattered fibroblastic phenotype along with decreased expression of E- and P-cadherin. Transfection of this cell line with antisense N-cadherin resulted in reversion to a normal appearing squamous epithelial cell with increased E- and P-cadherin expression. In addition, transfection of a normal-appearing squamous epithelial cell line with N-cadherin resulted in downregulation of both E- and P-cadherin and a scattered fibroblastic phenotype. In all cases, the levels of expression of N-cadherin and E-cadherin were inversely related to one another.  

Snug Woon PYO et al (2007) were suggested that reduced E-cadherin and positive N-cadherin expression are closely associated in oral squamous cell carcinoma, cadherin switching probably plays an important role in the development of oral squamous cell carcinoma and metastasis. 

Michelle E. Diamond et al (2008) were observed that, EGF and TGF-1 independently promoted migration of both oral keratinocytes and OSCC cells; EGF decreased TGF 1-mediated migration of oral keratinocytes but enhanced migration of OSCC cells. So EGF signaling has an important negative regulatory role on TGF 1-mediated N-cadherin expression and motility in normal oral keratinocytes, and in which loss of this regulatory mechanism accompanies malignant transformation of the oral epithelium. 

In oral squamous cell carcinoma, the nuclear pattern of N-Cadherin expression was particularly observed in dedifferentiated cancer, characterized by a worse prognosis (M. Di Domenico et al, 2011). Therefore the pattern of cadherin expression might constitute a useful diagnostic and prognostic tool in the evaluation of tumors and for determining the histogenesis of tumour cells. Moreover, they found a statistically significant correlation between N-Cadherin expression and grade, and a statistical trend for stage. 

Nguyen P T et al (2011) suggested that i) N-cadherin may play an important role in malignant behaviors of Head and neck squamous cell carcinoma (HNSCC) and ii) cadherin switching might be considered as a discrete critical event in EMT and metastatic potential of HNSCC. Kathryn R. Lawson and others (2006), suggested that the increased invasiveness seen in N-cadherin expressing cells of the oral squamous cell carcinoma are the result of N-cadherin-driven signaling pathways and not due to the associated loss of E-cadherin.

Phuong T and others (2011) study on spindle cell carcinoma suggested that N-cadherin may play an important role in metastasis of tumor in addition to the pathogenesis.

**N-cadherin expression in gastrointestinal cancer**

Study on esophageal squamous cell carcinoma (ESCC) by Ke Li et al (2009), suggested that, negativity of E-cadherin and positivity of N-cadherin were correlated significantly with both integrin-linked kinase (ILK) over expression and tumor metastasis. They suggested that ILK may have an important role in progression and metastasis of oral squamous cell carcinoma, possibly through EMT involving up-regulation of Snail and consequent aberrant expression of E-cadherin and N-cadherin.

Dan Zhao et al (2012) were observed the upregulation of Snail and N-cadherin and downregulation of E-cadherin correlated significantly with both integrin-linked kinase (ILK) over expression and tumor metastasis. They suggested that ILK may have an important role in metastasis of oral squamous cell carcinoma, possibly through EMT involving up-regulation of Snail and consequent aberrant expression of E-cadherin and N-cadherin.

Yingfeng Zhu and others (2007) were studied the
expression and significance of TGF-β1, Snail, E-cadherin and N-cadherin in Gastric Cancer (GC). The over-expression of TGF-β1 and Snail and decreased expression of E-cadherin and the abnormal expression of N-cadherin were involved in the process of invasion and metastasis of GC. The data showed that E-cadherin might switch to N-cadherin. TGF-β1 and Snail might play a fundamental role in the process.\(^{15}\)

Kunio Yanagimoto et al (2001) were studied the Co-expression of N-cadherin and α-fetoprotein (AFP) in stomach cancer. Expression of N-cadherin was observed in varying degrees in the intercellular spaces between tumor cells in 11 tubular adenocarcinomas and in six poorly differentiated adenocarcinomas, including E-cadherin-negative cases, all of which were AFP positive. The present findings suggested that a possible role for N-cadherin in gastric carcinoma.\(^{16}\)

Takahito Kamikihara et al (2012), also studied the neoexpression of N-cadherin in gastric cancer may be a useful prognostic marker independent of E-cadherin expression.\(^ {17}\)

Erika Rosivatz et al (2004), their study on human colon cancer by investigating the expression of N-cadherin and E-cadherin and their dependency on epithelial-mesenchymal transition regulators SNAI1, SIP1 and TWIST. They suggested that a mutual exclusion between abnormal E-cadherin reduction and upregulation of N-cadherin. For the first time, they postulate a role for N-cadherin in primary colon cancer progression.\(^ {18}\)

Zhuo HongQing et al (2013) also suggested that high N-cadherin expression may lead to tumor aggressiveness and metastatic potential in colorectal cancer, and may prove to be a possible prognostic factor.\(^ {19}\)

Sanae Nakajima and others (2004) were studied the N-cadherin expression in pancreatic carcinoma. N-cadherin expression correlated with neural invasion, histological type, fibroblast growth factor expression in primary tumors, and TGF expression and vementin in metastatic tumors. Their study provided morphological evidence of the occurrence of EMT in pancreatic carcinoma and found that overexpression of N-cadherin is involved in EMT and is affected by growth factors. Because EMT is an important process in the invasion and metastasis of malignant tumor cells, it is possible that N-cadherin is the adhesion molecule not only to acquire the fibroblastic morphology of EMT but also to obtain invasive and metastatic potential.\(^ {20}\)

The N-cadherin antagonist ADH-1 has significant antitumor activity against N-cadherin-expressing pancreatic cancer cells, both in vitro and in an orthotopic mouse model for pancreatic cancer (Yasushi Shintani et al, 2008). This study highly implicates N-cadherin as a valid target for treatment of human pancreatic cancer, and suggests that N-cadherin antagonists like ADH-1 that target its adhesive function should be developed for use in treatment of human pancreatic cancer.\(^ {21}\)

**N-Cadherin expression in Prostate cancer**

The expression of N-cadherin may in part play a role in the progression of prostate carcinoma from epithelium to mesenchyme; it is likely that N-cadherin mediates a less stable cell-cell adhesion and may allow for carcinoma cell invasion and stromal interactions(Figure 1). (Nhan L. Tran et al, 1999).

**Figure 1**

N-cadherin mediated stromal-mesenchymal cell adhesion.

N-cadherin mediates adhesion between β-catenin-deficient PC-3N (Prostate cancer) cells and stromal fibroblasts, which contain normal levels of all of the catenins. N-cadherin in
PC-3N cells may regulate the cellular outgrowth through cell-cell interactions, which may allow PC-3N to interact with surrounding prostate stromal fibroblasts. Meena Jaggi et al (2005), were demonstrated for the first time that N-cadherin switching occurs in higher grade prostate cancer and correlates significantly with increasing Gleason patterns. N-cadherin may be as a useful biomarker of aggressive prostate cancer.

Karsten Gravdal et al (2007), suggested the importance of epithelial to mesenchymal transition for prostate cancer progression, and demonstration of a switch from E-cadherin to N-cadherin expression could have significant effect on the care of prostate cancer patients. Androgen-deprivation therapy (ADT) is the standard treatment for metastatic prostate cancer. Karin Jennbacken and others (2010) investigated the expression of N-cadherin was influenced by androgen deprivation and was associated with metastasis in prostate cancer. Yuanyuan Cui and Soichiro Yamada (2013), found that formation of N-cadherin junctions promotes 3D (3 dimensional) cell migration of prostate cancer cells, and this is partly due to an aberrant regulation of the N-cadherin complex in the absence of α-catenin.

N-Cadherin expression in breast cancer

Marvin T. Nieman et al (1999) study suggested that N-cadherin promotes motility and invasion and that decreased expression of E-cadherin does not necessarily correlate with motility or invasion in breast cancer cells. In breast cancer, N-cadherin promotes motility, invasion, and metastasis even in the presence of the normally suppressive E-cadherin. The increase in MMP-9 production by N-cadherin expressing cells in response to a growth factor (FGF-2) may endow them with a greater ability to penetrate matrix protein barriers, while the increase in their adherence to endothelium may improve their ability to enter and exit the vasculature, two properties that may be responsible for metastasis of N-cadherin expressing cells (Rachel B. Hazan and others, 2000).

N-Cadherin expression in Lung cancer

Nakashima T and other’s (2003) study on non-small-cell lung cancer (NSCLC) suggested that, the frequency of hypervascular tumours was significantly higher for N-cadherin-positive carcinomas than for N-cadherin-negative carcinomas and the 5-year survival rate of patients with N-cadherin-positive tumours was significantly lower than that of patients with N-cadherin-negative tumours.

Specific tyrosine kinase inhibitors for epidermal growth factor receptor (EGFR), such as gefitinib, have been effective in some NSCLC (Non-small cell lung cancer) patients and are being used in the clinical setting as pioneer molecularly targeted cancer drugs. However, many patients have not responded to these drugs, and have acquired resistance after long-term treatment. Mai Yamauchi et al (2011) suggested that, N-cadherin maintains the survival of the gefitinib-resistant lung cancer cells via the PI-3 kinase/Akt survival pathway. So N-cadherin is a potential molecular target in the treatment of NSCLC. The overexpression of Twist and N-cadherin could be considered as useful biomarkers for predicting the prognosis of NSCLC. Twist1 could inhibit apoptosis and promote the invasion of lung cancer cells, and depletion of Twist1 in lung cancer cells led to inhibition of N-cadherin expression (Linping Hui et al 2013).

Xiaoju Zhang et al (2013), study reveals that the upregulation of N-cadherin in H1650ER (Erlotinib-resistant cell line) cells leads to increased tumor cell migration, invasion and tumorigenic potential. Their results also suggested that the maintenance of the EMT phenotype in H1650ER cells may be related to the sustained expression of N-cadherin. Therefore, N-cadherin may serve as a promising
new target for the treatment of cancers with acquired resistance to EGFR-TKIs (Epidermal growth factor receptor-Tyrosine Kinase Inhibitors).34

N-Cadherin expression in urinary system cancer

Tani T et al (1995), were studied the expression of E- and N-cadherin in renal cell carcinoma. Normally kidney tubules express these molecules in a distinctive pattern, the expression of N-cadherin being restricted to proximal tubules and that of E-cadherin to distal tubules and collecting ducts. Their study suggested that most renal cell carcinomas (RCCs) co-express the characteristic adhesion molecules of both proximal and distal tubules, which makes it questionable whether the origin of these tumors can be reliably located to any distinct part of the renal tubule.35 Toru shimazui et al (2005), suggested that N-cadherin plays a different role from E-cadherin or cadherin-6 in RCC and may be associated with the aggressiveness and malignant potential of RCC.36

Carl Ludwig Behnes et al (2012) were observed the N-cadherin expression in histological subtypes of papillary renal cell carcinoma and N-cadherin represents the first immunohistochemical marker for a clear cut differentiation between papillary RCC type I and type II and could be a target for therapy and diagnostic in the future.37

N-cadherin was present at cell-cell borders in the very anaplastic cell lines of human bladder carcinoma, observed by Mialhe A and others (2000) and they were indicated that N-cadherin may participate in intercellular adhesion, while facilitating bladder tumorigenesis.38 in bladder cancer, loss or reduced E-cadherin expression has been associated with poor survival, and aberrant expression of N-cadherin has been associated with the invasive phenotype of bladder carcinoma cells. Isabelle Lascombe et al (2006), were studied the N-cadherin in Superficial Urothelial Tumors. N-cadherin expression was absent in normal urothelium, appeared in stage pT1, and increased in pT2-pT3 tumors. In most cases, increased N-cadherin expression in invasive tumors was associated with loss of E-cadherin expression. Importantly, this study identified N-cadherin as a novel prognostic marker of progression in superficial urothelial tumors.39 Richard T. Bryana and Chris Tselepisa (2010), were suggested that Cadherin switching is an important process late in the molecular pathogenesis of bladder cancer.40

In summary, oral squamous cell carcinoma with high percentage of N-cadherin expression was associated with a worse clinical outcome, reduced overall survival and a major tendency for loco-regional invasion and metastasis. So the increased expression of N-cadherin is associated with high grade malignancy and poor prognosis and since N-cadherin can play an important role in oral carcinogenesis. In esophageal squamous cell carcinoma N-cadherin expression was negatively correlated with E-cadherin expression and it is associated with invasion, differentiation and metastasis. In gastric cancer also some of the study suggested the same, abnormal expression of N-cadherin expression were involved in invasion and metastasis. In colorectal cancer, high N-cadherin expression may lead to tumor aggressiveness and metastasis. And in pancreatic cancer suggested that, N-cadherin is an important marker for EMT; it is an important process in the invasion and metastasis. In prostate cancer, N-cadherin mediates less stable cell-cell adhesion and it is favorable to stromal interaction and invasion of carcinoma cell. And also suggested that, EMT is directly related to cadherin switch from E-cadherin to N-cadherin. In breast cancer, increased expression of N-cadherin promotes motility and invasion of the tumor cells even with the presence of normal E-cadherin. And some of the studies suggested that over expression of N-cadherin were significantly correlated with poorly differentiated carcinoma, positive node status, and poor Nottingham Prognostic Index. In Lung cancer, N-cadherin expression was significantly correlated with hypervascular tumors and less survival rate of the patient. Some of the study found that maintenance of the EMT phenotype in lung cancer cells may be related to sustained expression of N-cadherin. N-cadherin may be associated with the aggressiveness and malignant potential of renal cell carcinoma. And it may be the important immunohistochemical marker for a clear cut differentiation between papillary RCC type I and type II. And in human bladder carcinoma N-cadherin may involved in intercellular adhesion, while facilitating bladder tumorigenesis.

CONCLUSION

Expression of N-cadherin may in part play a role in the progression of carcinoma from epithelium to mesenchyme, it is likely that N-cadherin mediates a less stable cell-cell adhesion and may allow for carcinoma cell invasion and stromal interactions. The loss of E-cadherin expression and gain of N-cadherin expression is reminiscent of the cadherin switching that is seen during epithelial to mesenchymal transition. Cancer cells derived from epithelium inappropriately express N-cadherin, and the up regulation of
N-cadherin expression has been shown to promote motility and invasion.

References

27. Marvin T. Nieman, Ryan S. Prudoff, Keith R. Johnson, Margaret J. Wheelock. N-Cadherin Promotes Motility in


33. Linping Hui, Siyang Zhang, Xinjun Dong, Dali Tian, Zeshi Cui, Xueshan Qi. Prognostic Significance of Twist and N-Cadherin Expression in NSCLC. PLOS One. April 2013, Volume 8, Issue 4.

34. Xiaoju Zhang, Guangzhi Liu, Yi Kang, Zhaogang Dong, Qiyu Qian, Xitao Ma. N-Cadherin Expression Is Associated with Acquisition of EMT Phenotype and with Enhanced Invasion in Erlotinib-Resistant Lung Cancer Cell Lines. PLOS One. March 2013, Volume 8, Issue 3.


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