E-kadherin in β-katenin: dvojna vloga v karcinogenezi

E-cadherin and β-catenin: Dual roles in carcinogenesis

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Izvleček

V urejanje epitelno-mezenhimskega prehoda in obnavljanje epitelnih tkiv so vključeni strogo določeni kontrolni mehanizmi, njihova odsotnost pa lahko vodi do karcinogeneze. Ti določeni procesi obsegajo regulacijo genske ekspresije in celičnega citoskeleta do proliferacije in apoptoze. Glavno vlogo imata E-kadherin in β-katenin, molekuli, ki sta sestavna elementa med celicami. β-katenin je ključni posrednik na Wtn poti, ki regulira ekspresijo različnih proteinov, zajetih v morfogenezo, in celično preživetje preko TCF/LEF poti. Prispevek opisuje ekspresijo E-kadherin in fosfataz kot negativen regulator Wnt poti, kar omogoča diferenciacijo, medtem ko tirozin kinaza poveča Wtn pot preko fosforilacije kadherinov in kateninov, kar povzroči povečano proliferacijo in inhibicijo apoptoze.

Abstract

Strict signalling control mechanisms have evolved to regulate epithelial–mesechymal transitions and regeneration of epithelial tissues, and failure of these processes may lead to carcinogenesis. These signalling processes range from regulation of gene expression and cell cytoskeleton to proliferation and apoptosis, and the central players include E-cadherin and β-catenin. These molecules function as structural elements at cell–cell contact sites and β-catenin is the key intermediate in the Wnt signalling pathway, which regulates gene expression of various proteins involved in morphogenesis and cell survival via the TCF/LEF pathway. The present review describes how the expression of E-cadherin and phosphatases act as negative regulators of Wnt signalling, promoting differentiation, whereas tyrosine kinases amplify the Wnt signalling pathway via phosphorylation of cadherins and catenins, resulting in increased proliferation and inhibition of apoptosis.

INTRODUCTION

Determination of the cell signalling mechanisms that control cell proliferation and differentiation has had a great impact on the development of cancer treatment. Especially, understanding of the central role of tyrosine kinases in these processes has led to intensive research and the development of several tyrosine kinase inhibitors, currently used in the treatment of leukemias, lymphomas and breast cancer. The number of these inhibitor molecules that have reached clinical trials has grown exponentially in the past ten years. Another set of molecules under intensive research are inhibitors of apoptosis, and these are expected to reach clinical trials in the near future. The majority of human cancers are of epithelial origin and therefore the mechanisms controlling epithelial–mesenchymal transition (EMT) and cell migration are the focus of much cancer research with great expectations for the development of new therapeutic approaches.

METHODS

Carcinogenesis

Epithelial cells: boundary to external milieu

Extensive remodelling of tissues occurs during embryogenesis and carcinogenesis, and in some diseases like fibrosis or during the process of wound healing. Epithelial cells that delineate tissues and glands and separate them from the environment have an important task in these remodelling events. They must maintain their polarized structures and transport properties throughout their lifespan, and the cell–cell junctions should not disintegrate during cell proliferation and reorganization. The mechanisms to control cell adhesion in tissues range from gene expression to cell proliferation and apoptosis, and include cadherins and associated proteins; catenins are considered key players in these processes. In addition to cell–cell contacts, integrin–mediated contacts to extracellular matrix components are crucial for the differentiation of epithelial cells.

In understanding differentiation and tissue regeneration it is important to identify the signalling pathways that coordinate changes in gene expression linked to dynamic changes in cell adhesion and migration. Loss of control of these pathways will alter the cell behaviour, leading to carcinogenesis, characterized by increased migration and proliferation and reduced adhesion and apoptosis (1). Evolution is an economical process and the same proteins involved in adhesion have a central function in cell signalling processes that regulate gene expression. Two major components in regulating adhesion during differentiation and transcription during malignant transformation are E–cadherin and β–catenin. Only after identification of the Wnt signalling pathway was the link between the dynamic interaction of β–catenin with E–cadherin and the transformation process determined (2, 3). The Wnt gene had originally been identified in *Drosophila melanogaster* as a gene that affects wing development and was later found to represent a group of secreted small proteins acting as universal regulators of morphogenesis (2). Lymphoid enhancer factor–1 (LEF–1) is a cell–type specific transcription factor that, together with other transcription factors termed LEF–1, TCF–1, TCF–3, and TCF–4, has an important role in the Wnt signal transduction pathway directing cell differentiation. The LEF/TCF (T cell factor/lymphoid enhancer factor) forms complexes with β–catenin that interact with the specific sequence in the promoter region and results in transcriptional activation of the target gene.

The Wnt signalling pathway

The canonical Wnt signalling pathway is highly conserved and broadly employed in multicellular organisms. Wnts operate beyond cell or tissue boundaries, as they are secreted from one cell or tissue type to activate surface receptors, signal transduction components and transcription factors in the neighbouring cells or tissues, regulating processes such as cell proliferation, survival or differentiation. The central player in the Wnt pathway is β–catenin, which is a transcription co-factor with TCF/LEF and, at the same time, acts

as a structural adaptor protein linking cadherins to the actin cytoskeleton in adherent epithelial cells (3). In cells, β–catenin functions as an intermediary signal in the Wnt pathway. In the absence of a Wnt signal, the cytoplasmic β–catenin level is maintained at low levels via rapid degradation by a protein complex containing APC (adenomatous polyposis coli), axins and GSK3β (glycogen synthetase kinase 3β), which targets it to the proteosome for degradation. Binding of the Wnt ligand to the extracellular domains of two transmembrane receptors leads to inhibition of the activity of the destruction complex, resulting in accumulation of high levels of β–catenin in the cytoplasm. Increased amounts of β–catenin, in conjunction with TCF/LEF transcription factors, activate the transcription of specific target genes, such as a regulator of cell division, cyclin D1, an apoptosis inhibitor survivin and the proto–oncogene MYC (2, 3).

More than 90% of colon cancers and a high percentage of other cancers originate from activating mutations in the Wnt pathway. Mutated proteins linked to cancer include APC, axins and β–catenin (3, 4). Cell culture studies have shown that inhibition of the Wnt pathway can normalize cancer cells, that is, inhibit proliferation and induce differentiation. Therefore, it is likely that inhibiting the Wnt pathway could make a contribution to cancer therapy. On the other hand, loss of the physiological function of E–cadherin by mutation or transcriptional silencing is associated with tumour cell invasion and metastasis *in vitro* and in tumours, indicating the importance of E–cadherin loss in tumour progression (5). Therefore one might expect that the fate of two molecules, E–cadherin and β–catenin, might cross in search of a balance between differentiation and proliferation.

Functional links between E–cadherin and β–catenin

There is a close link between expression and localization of E–cadherin and catenins in epithelial cells. At adherens junctions, β–catenin binds tightly to the cytoplasmic domain of E–cadherin and has an essential role in the structural organization and function of cadherins by linking them through α–catenin to the actin cytoskeleton. Another catenin, p120catenin, binds to the membrane proximal domain of cadherin and further regulates the structural integrity and function of the cadherin complex. Hence, one might expect that integrity of adherens junctions and Wnt signalling are physically and functionally linked in the regulation of gene expression (4). This link could be mediated by a shuttling of β–catenin between the adhesion complex and cytoplasm. In this way, E–cadherin could have an inhibitory role in the TCF/LEF pathway through the sequestration of β–catenin at the plasma membrane. There are reports that down–regulation of E–cadherin expression by up–regulation of a cadherin repressor, Snail, activates β–catenin/TCF–dependent transcription in colon tumour cells and this activation can be reversed by over–expressing E–cadherin (6).

The presumption that cadherin loss automatically promotes β–catenin signalling by releasing it from the cadherin–bound pool is largely incorrect (4). *In vitro* experiments in which E–cadherin or β–catenin, alone or simultaneously, have been knocked down in cultured cells have shown that the loss of E–cadherin can induct EMT, invasiveness, and resistance to anoikis (apoptosis due to lack of matrix contact), all of which are common in cancer cells (5). In these processes, the presence of β–catenin is necessary, but its over–expression is not sufficient to induce all these events (5). There are other signalling mechanisms needed for malignant transformation and other, β–catenin–independent transcription factors, such as Twist, seem to act in parallel with β–catenin to program the cellular changes observed after E–cadherin loss (5). There is evidence that fold–changes, and not absolute levels, of cytoplasmic β–catenin controls Wnt signalling (7).

Inhibition of inhibition: p120catenin and Kaiso

Another component of adherens junctions, p120catenin also functions in Wnt signalling. It was first identified as a substrate of the Src onco-

gene, and subsequently as a component of adherens junctions, where it binds E–cadherin and acts as a stabilizer of junctions. It has a binding partner, Kaiso, which belongs to the zinc finger protein family that has been implicated in tumorigenesis (8, 9). Kaiso was first discovered in a complex with p120catenin and has since been shown to function as a transcriptional repressor. Like β–catenin, p120catenin and Kaiso shuttle between the cytoplasm and the nucleus. Kaiso seems to associate with LEF/TCF and inhibit binding of β–catenin to LEF/TCF, providing a mechanism by which repression of some canonical Wnt gene targets can be increased. In turn, nuclear p120catenin can dissociate Kaiso from the binding sites, which allows activation of the TCF/LEF target genes. Thus, both catenins have a role in Wnt signalling, but p120catenin acts by inhibiting Kaiso DNA–binding, whereas β–catenin behaves a coactivator but does not affect TCF DNA binding. Decreased levels or the absence of E–cadherin expression, as found in numerous tumour cell lines and cancers, is thought to free p120catenin from cell–cell junctions. Such cytoplasmic p120catenin might then enter the nucleus to relieve Kaiso–mediated gene repression. Nuclear p120catenin is, however, transient and heterogeneous. The significance of the convergence of p120catenin/Kaiso and Wnt/ β–catenin pathways in mammals is not yet known.

Kinases and phosphatases direct E–cadherin and catenins from and to the plasma membrane

In theory, malignant transformation, increased proliferation, cell migration and inhibition of apoptosis are promoted by tyrosine kinases, whereas stabilized cell–cell adhesion, formation of proper apico–basal axis and improved function of epithelium is promoted by protein tyrosine phosphatases.

Stability of E–cadherin–mediated adherens junctions is negatively regulated by the actions of several tyrosine kinases in tumour cells (1). Cell surface levels of E–cadherin are regulated by endocytosis through clathrin–mediated, caveolae–mediated or lipid–raft–mediated internalization routes, as well as by macropinocytosis. Phosphorylation of the intracytoplasmic tail of E–cadherin by receptor (EGFR and c–Met) or nonreceptor tyrosine kinases (Fyn and c–Src) promotes its binding to Hakai, an E3 ubiquitin ligase, resulting in loss of E–cadherin and disruption of adherens junctions, thereby inducing EMT (1). Also, β–catenin and p120catenin are highly tyrosine phosphorylated in v–Src–transformed cells and phosphorylation of β–catenin results in dissociation of α–catenin from β–catenin, a feature that strongly correlates with defective E–cadherin–mediated adhesion (9, 10). These events can be reversed by inhibiting tyrosine kinases by chemical reagents or tyrosine phosphatases.

Another type of phosphatase, a lipid phosphatase, is PTEN (phosphatase and tensin homologue deleted from chromosome ten). It degrades the second messenger phosphoinositol(3,4,5) phosphate (PIP3) to phosphoinositol(4,5)phosphate (PIP2), thereby preventing growth factor receptor stimulation and interrupting downstream signaling (11). The PI3 kinase–Akt kinase pathway has a central role in the control of cell proliferation, growth and survival, and PTEN is the only negative regulator of the pathway identified so far. Genetic mutation or deletion of PTEN is so common in many types of human cancers that it can be considered one of the most commonly mutated tumour suppressor genes (12). There is a close link between PTEN and E–cadherin/β–catenin function, and E–cadherin seems to regulate both PTEN protein levels and its recruitment to cell–cell junctions of mammary epithelial cells (13). In turn, nuclear β–catenin expression and TCF transcriptional activity are constitutively increased in PTEN–null prostate cancer cells and the expression of PTEN inhibits nuclear localization of β–catenin, suppresses TCF transcriptional activity and induces E–cadherin expression (14). A summary of the signalling cascades involved in regulation of TCF/LEF transcription is shown in the Figure.

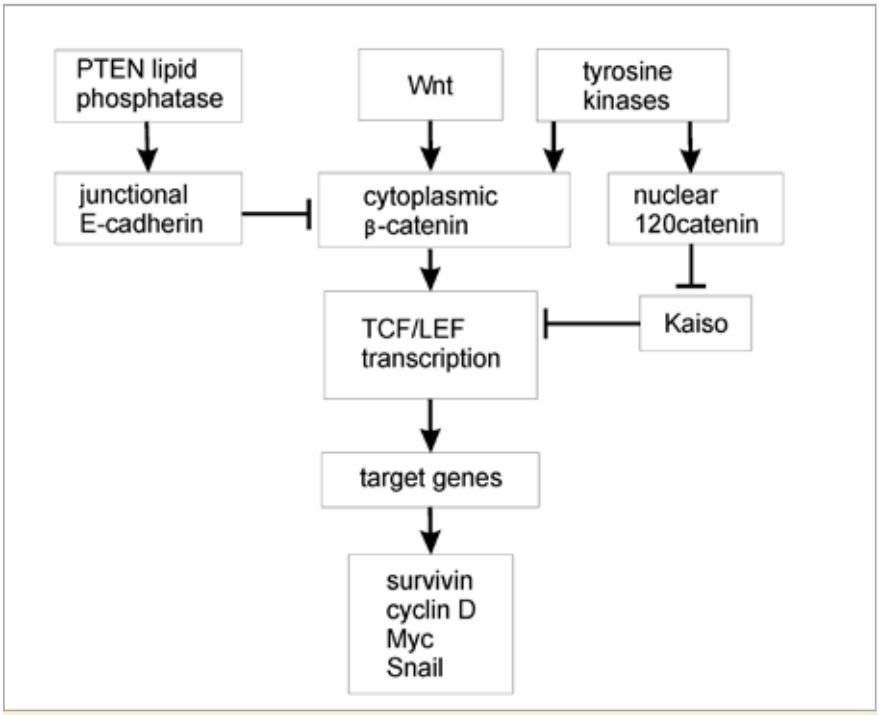

Figure. *Summary of the signalling pathways controlling TCF/LEF transcription. Activated Wnt signalling stabilizes cytoplasmic β–catenin and activates TCF/LEF transcription factors, resulting in transcription of target genes. Lipid phosphatase PTEN promotes expression and junctional localization of E–cadherin; junctional E–cadherin, in turn, sequesters β–catenin and p120catenin to cell–cell adhesion sites and inhibits TCF/LEF transcription. The p120catenin associated protein, Kaiso, also represses transcription, but this repression can be relieved by nuclear localization of p120catenin. Receptor or non–receptor tyrosine kinases phosphorylate β–catenin and p120catenin and disintegrate adherens junctions, thereby releasing catenins from the junctions amplifying Wnt signalling.*

Down–stream signals of the TCF/ β–catenin pathway

Several genes involved in carcinogenesis are targets of the TCF/ β–catenin signalling patway. An updated list is available at the Wnt homepage created by Roel Nusse (http://www.stanford.edu/~rnusse/pathways/targets.html). Examples of upregulated genes include the oncogenes c–Myc and Met, the matrix metalloproteinase MMP–7, the regulator of cell division cyclin D, the transcription factors TCF–1 and LEF1, the apoptosis inhibitor survivin, and the E–cadherin repressor Snail; in contrast, E–cadherin itself is down–regulated. One of the targets, survivin, has received considerable attention in the search for new cancer therapies because it is strongly expressed in almost all cancers and undetectable in most adult tissues (15). Survivin is an essential regulator of cell division, a modulator of apoptotic and non–apoptotic cell death, and a stress response factor ensuring continued cell proliferation and cell survival in unfavourable milieus. Survivin expression in cultured cells is promoted by v–Src and suppressed by PTEN, supporting the hypothesis that several signalling pathways amplify each other either in maintaining the polarized epithelial phenotype or in facilitating the transformation process during carcinogenesis (11, 16).

CONCLUSIONS

Extensive research has focused on identifying inhibitors of survivin, but these efforts have not yet led to the development of successful therapeutic agents. PTEN is the most commonly mutated tumour suppressor gene and identifying a PTEN inhibitor appears even more difficult. Gene therapy is the next great challenge in cancer research. The development of methods to replace mutated PTEN genes with a normal gene or to silence the survivin gene in cancer tissues might lead to a great breakthrough in treatment of carcinomas in the same way that tyrosine kinase inhibitors have helped in the treatment of leukemias and lymphomas.

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