

# Genetic And Epigenetic Regulation Of E-Cadherin Signaling In Human Hepatocellular Carcinoma

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**Abstract:** E-cadherin is well known as a growth and invasion suppressor and belongs to the large cadherin family. Loss of E-cadherin is widely known as the hallmark of epithelial-to-mesenchymal transition (EMT) with the involvement of transcription factors such as Snail, Slug, Twist and Zeb1/2. Tumor cells undergoing EMT could migrate to distant sites and become metastases. Recently, numerous studies have revealed how the expression of E-cadherin is regulated by different kinds of genetic and epigenetic alteration, which are implicated in several crucial transcription factors and pathways. E-cadherin signaling plays an important role in hepatocellular carcinoma (HCC) initiation and progression considering the highly mutated frequency of CTNNB1 (27%). Combining the data from The Cancer Genome Atlas (TCGA) database and previous studies, we have summarized the roles of gene mutations, chromosome instability, DNA methylation, histone modifications and non-coding RNA in E-cadherin in HCC. In this review, we discuss the current understanding of the relationship between these modifications and HCC. Perspectives on E-cadherin-related research in HCC are provided.

**Keywords:** E-cadherin, HCC, genetic alterations, epigenetic alterations

## Introduction

Liver cancer is highly prevalent in Asia, and especially in China. It is estimated to be the sixth most commonly diagnosed form of cancer and the fourth cause of cancer-related death in 2018.<sup>1</sup> Hepatocellular carcinoma (HCC) is a typical liver cancer, accounting for approximately 70–85% of all cases.<sup>2</sup> Recent studies have revealed that HCC development is the result of an accumulation of genetic and epigenetic alterations, which are implicated in several crucial pathways and processes, such as the cadherin pathway.

There are at least nine subfamilies of cadherin involved in cancer, as follows: classical type I (represented by E-cadherin CDH1); classical type II (represented by CDH11); desmosomal cadherin (represented by DSG2); seven-domain (7D) cadherin (CDH16 and CDH17); truncated cadherin (also known as T-cadherin); clustered protocadherins (PCDHs), represented by PCDH6; non-clustered protocadherin is divided into two subfamilies which are called the  $\delta 1$  subfamily and  $\delta 2$  subfamily; and the cadherin-related protein (represented by FAT1, FAT4).<sup>3</sup>

E-cadherin (CDH1) is reported to be a key growth and invasion suppressor in cancer. The mature E-cadherin protein in humans consists of a single transmembrane domain, and a cytoplasmic domain, which connects with the catenin complex, and five cadherin repeats in the extracellular domain, named EC1–EC5.<sup>4,5</sup> The

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EC1 subdomain differs from the others in that it contains a histidine-alanine-valine sequence, which is triggered by the binding of calcium at the EC1–EC2 interface to create the trans dimerization which is essential for adhesion between adjacent cells.<sup>6</sup> As for the cytoplasmic domain, early studies have proposed a model for an E-cadherin/ $\beta$ -catenin complex (CCC).<sup>7,8</sup> The cytoplasmic domain of E-cadherin helps to establish cell–cell adhesion through binding to the cytoskeleton via the catenin protein family containing  $\alpha$ -catenin,  $\beta$ -catenin and  $\gamma$ -catenin.<sup>9</sup> Besides E-cadherin and catenin, there are also other proteins, for example the P120 arm-family protein, that act as key regulators of the E-cadherin/ $\beta$ -catenin complex.<sup>10,11</sup> In the current review, we focus on the genetic and epigenetic regulatory mechanisms involved in E-cadherin signaling in cancers, mainly in HCC.

## The Function And Detection Of E-Cadherin In Cancer

E-cadherin has received increasing attention in the past few years because of its extensive impact in human cancers, such as gastric cancer,<sup>12,13</sup> breast cancer,<sup>14</sup> HCC<sup>15</sup> and renal cell carcinomas.<sup>16</sup> In the progression of human cancer, loss of E-cadherin is widely known as the hallmark of epithelial-to-mesenchymal transition (EMT), which is considered to be a strong signal of tumor progression and metastasis.<sup>17</sup> Tumor cells undergoing EMT act as cancer stem-like cells, migrate to distant sites and become metastases.<sup>18</sup> Contact inhibition of locomotion (CIL) is a phenomenon whereby cells cease moving after cell–cell contact. The adhesive properties of E-cadherin hold healthy cells together and maintain CIL. However, because of the dysregulation of E-cadherin in malignant cells, CIL is disrupted which facilitates cancer cell migration to distant sites.<sup>19,20</sup> Contact inhibition of proliferation (CIP) refers to a phenomenon in which the ability of cell proliferation is affected by cell density.<sup>19</sup> CIP is important for maintaining tissue morphogenesis. However, when CIP is disrupted because of the loss of E-cadherin, it can lead to uncontrolled growth and tumor initiation. Although the underlying regulatory mechanism for CIP remains unclear, some mechanisms have been proposed to explain it. For example, the E-cadherin extracellular domain is thought to bind to receptor tyrosine kinases, including the EGF receptor (EGFR) and c-Met, to decrease rates of cell growth.<sup>21,22</sup> Besides migration and proliferation, E-cadherin is also reported to

affect cell contraction through the cadherin–actomyosin system.<sup>23</sup>

The comprehensive biological functions of E-cadherin have been partly attributed to the interaction between E-cadherin and multiple signaling pathways, such as the Wnt/ $\beta$ -catenin pathway, the PI3K/AKT pathway, the hippo pathway and the NF $\kappa$ B signaling pathway.<sup>24,25</sup> E-cadherin in densely packed epithelial monolayers can inhibit the access of EGF to the EGFR as well as down-stream signaling from the EGFR via Merlin.<sup>22</sup> However, E-cadherin is also regulated by numerous pathways. Studies in a variety of cancers have documented the complex relationship between  $\beta$ -catenin signaling and E-cadherin-mediated cell–cell adhesion. Nuclear  $\beta$ -catenin signaling plays a central role in human cancer,<sup>26</sup> including prostate cancer,<sup>27</sup> breast cancer,<sup>28</sup> colon cancer<sup>29</sup> and HCC.<sup>30</sup> There is a well-established model which demonstrates how  $\beta$ -catenin is involved in cancer initiation, progression, dormancy, immunity and cancer stem cell maintenance.<sup>31</sup> First, a reduction in cadherin levels can release  $\beta$ -catenin bound at the cell surface. As a result, nuclear  $\beta$ -catenin signaling is enhanced.<sup>32</sup> However, reduced E-cadherin levels do not always lead to constitutive  $\beta$ -catenin signaling activation.<sup>33</sup> The  $\beta$ -catenin-TCF has been reported to regulate the Axin2-GSK3 $\beta$ -Snail1 axis to influence the expression of E-cadherin.<sup>34</sup> In vitro, the E-cadherin promoter region was found to be bound by LEF-1/ $\beta$ -catenin complexes, which indicates a negative feedback loop in Wnt/ $\beta$ -catenin signaling and E-cadherin expression.<sup>35</sup> The complex link between E-cadherin and  $\beta$ -catenin signaling requires further exploration in future research.

The expression of E-cadherin can be detected at the transcriptional level by monitoring CDH1 mRNA levels, or directly at the protein level. RT-qPCR is widely used for mRNA detection.<sup>36</sup> In addition, RNA-seq<sup>37</sup> and probes<sup>38</sup> are also frequently used to detect mRNA. Several methods have been employed for the detection of proteins, including Western blotting, immunohistochemistry and immunofluorescence methods. E-cadherin as a transmembrane linker protein sometimes is hard to be detected because of the hydrophobicity of membrane proteins. To solve this problem, nanodiscs for solubilizing membrane proteins have been developed.<sup>39</sup> With its help, the traditional detection techniques, such as electron microscopy, small-angle X-ray scattering and mass spectroscopy method, could detect E-cadherin more easily. Interestingly, researchers have developed a novel electrochemical label-free immunosensor to identify the expression of E-cadherin by

modifying indium-tin oxide electrodes with anti-E-cadherin monoclonal antibodies.<sup>40</sup>

## The Impact Of Genetic Alterations On E-Cadherin Signaling Regulation

Cancer progression has long been regarded as an outcome of genetic alterations. Point mutations, deletions, insertions, amplifications and translocations in oncogenes and suppressors are believed to be responsible for HCC development.<sup>41</sup> As an important factor in HCC progression, genetic alterations also play a large role in E-cadherin signaling regulation.

### Gene Mutations

The cluster of the CDH1 is located at chromosome 16q22.1. Researchers have identified its structure using cDNA probes covering the whole protein-coding sequence. The whole length of the CDH1 gene contains 16 well-conserved exons ranging in size between 115 and 2245 bp and 15 introns ranging from 120 bp to 65 kb.<sup>42</sup> Recently, germ-line mutation of CDH1, which means any detectable variation within germ cells, has been found in hereditary diffuse gastric cancers<sup>13</sup> and lobular breast cancer.<sup>43</sup> A total of 69 different kinds of CDH1 somatic mutation have been reported. The mutation types mainly consist of splice site mutations and truncation mutations which are caused by insertions, deletions, and nonsense mutations.<sup>44</sup> These kinds of mutation have been found to commonly occur in lobular breast cancer<sup>45</sup> and diffuse gastric cancer,<sup>46,47</sup> but rarely in HCC.<sup>44,48</sup> In most cases, these mutations, which occur in combination with a loss of heterozygosity (LOH) of the wild-type allele, have been reported to downregulate the expression of E-cadherin in HCC. Betty L. et al, observed 64% of allele loss for CDH1 in hepatitis B virus-positive Chinese HCC patients.<sup>49</sup> Yu et al, analyzed the retained E-cadherin allele in nine tumor samples harboring LOH in both 16q and 16p markers and detected a silent polymorphism at codon 692 (GCC→GCT) in two samples, without any other nucleotide changes.<sup>50</sup> These findings indicate that the mechanism of E-cadherin inactivation in hepatoma is more likely attributable to the loss of one copy of the E-cadherin gene rather than the mutation of the CDH1 gene itself.

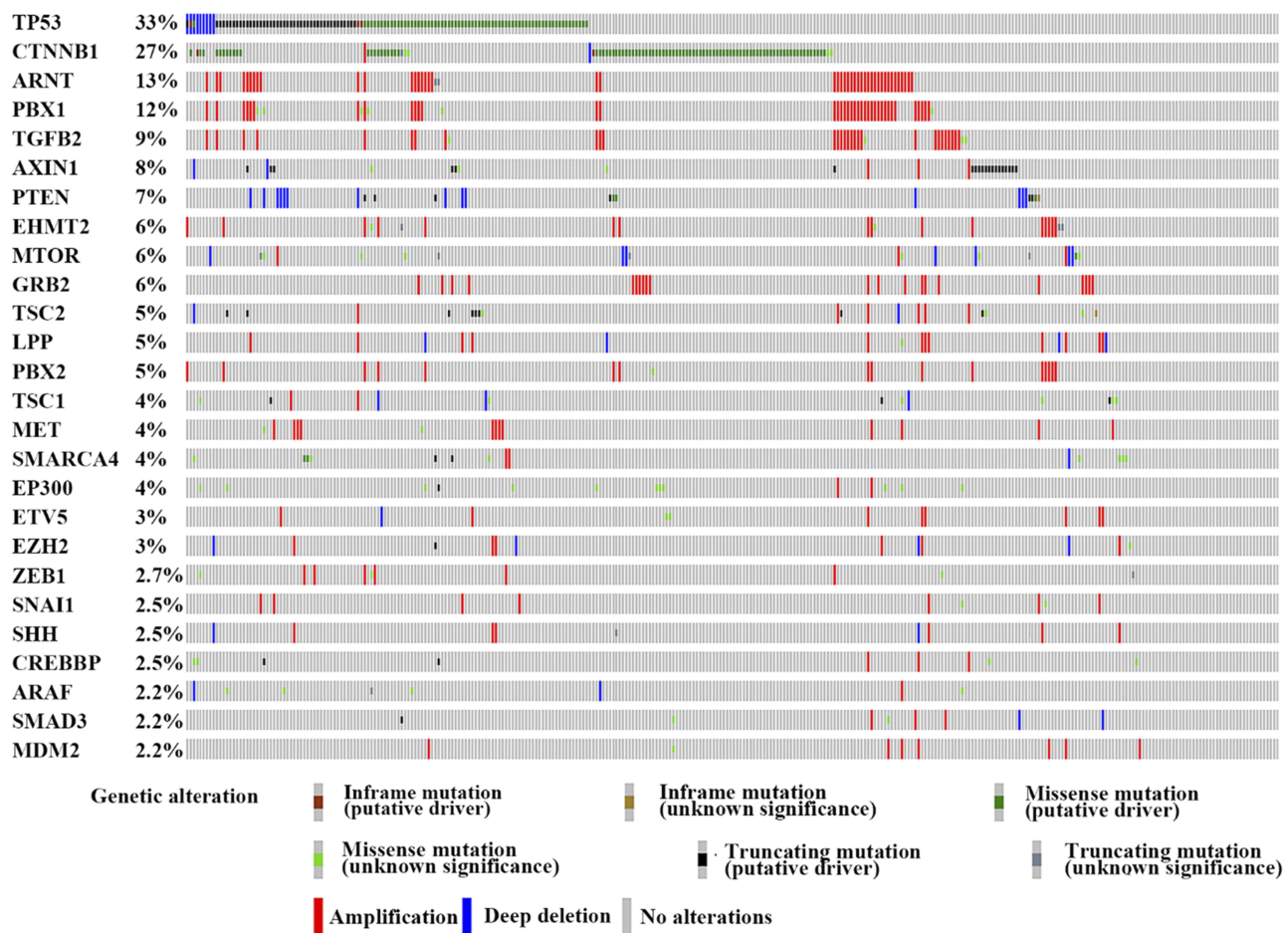
To clarify the genetic network of E-cadherin expression, we systematically reviewed previous studies and found 71 genes which were reported to be significantly related to E-cadherin regulation. We consulted the Cancer

Genome Atlas (TCGA) database and found that 26 genes were frequently altered (>2%) in HCC. These 26 genes are presented in Figure 1.<sup>51</sup>

The most frequently altered gene was P53. The genetic alteration of this gene was observed in HCC in approximately 33% of clinical cases. One SNP array analysis of 125 HCC patients revealed that 20.8% of TP53 mutations were inactivating mutations.<sup>52</sup> Recently, it was found that TP53 mutant cell lines showed a smaller cell size in a hepatocellular cancer spheroid formation test, which resulted from the lower CDH1 expression levels.<sup>53</sup> Moreover, evidence demonstrated the mutant-p53 could affect the expression of Twist1 and Smad interacting protein 1 (SIP1) and further regulate E-cadherin in adrenocortical carcinoma.<sup>54</sup> In ovarian tumor cells, p53 has been reported to repress E-cadherin expression by recruiting DNA (cytosine-5)-methyltransferase 1 (DNMT1) to the CDH1 promoter through activation of the PI3K/Akt pathway.<sup>55</sup>

CTNNB1 encodes for  $\beta$ -catenin and is another high-frequency mutation gene in HCC and primarily demonstrates missense mutations. The Wnt- $\beta$ -catenin pathway is often activated in HCC through CTNNB1 mutations (27% of HCC cases in TCGA data set, Figure 1) which is believed to have a strong impact on E-cadherin expression (Figure 2).<sup>56,57</sup> In a study of renal interstitial fibrosis (RIF), the expression of E-cadherin was repressed through downregulation of miRNA-200a which directly targets the CTNNB1 3'UTR.<sup>58</sup> In a study of PS341 (Bortezomib), which can inhibit the transcriptional activation of CTNNB1, Yang et al, reported a positive relationship between E-cadherin and PS341 levels.<sup>59</sup> As the encoding gene of  $\beta$ -catenin, CTNNB1 can change the expression of E-cadherin via altered  $\beta$ -catenin protein levels. There is, however, a lack of direct evidence to clarify the impact of different CTNNB1 mutation types on E-cadherin expression in HCC.

AXIN1 has been identified to be a concentration-limiting factor responsible for the destruction of  $\beta$ -catenin. In the Wnt/ $\beta$ -catenin pathway, when Wnt does not bind to the receptor, AXIN, GSK and APC form a destruction complex to accelerate the destruction of  $\beta$ -catenin. Conversely, when the Wnt pathway is active, AXIN is removed from the destruction complex which results in  $\beta$ -catenin moving into the nucleus and binding to target DNA to activate transcription. However, we had to emphasize that previous studies have also indicated that CTNNB1 and AXIN gene alterations are mutually exclusive.<sup>52</sup> Zucman-Rossi et al,



**Figure 1** The genetic alteration of the genes involved in the E-cadherin regulation process. We proceeded to the TCGA database and finally identified 26 genes which were frequently altered (>2%) in HCC.

**Notes:** This figure is from the cBioPortal website (<http://www.cbioportal.org>); TCGA data set. Cerami E, Gao J, Dogrusoz U, et al The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2012;2(5):401–404.<sup>51</sup> Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal.* 2013;6(269):p11.<sup>211</sup>

suggested that the loss of function of AXIN1 is not equivalent to the gain of function of  $\beta$ -catenin in HCC.<sup>60</sup> In view of the complex network of AXIN relationships, more research on this topic is required.

## Chromosome Instability

Chromosome instability (CIN) is an interesting aspect of genetic variation in CDH1. CIN is identified as the rate (cell to cell variability) of changed karyotypes of a given cell population, including gaining or losing whole chromosomes or fractions of chromosomes or altering the structure of chromosomes.<sup>61</sup> Substantial evidence has demonstrated the participation of aneuploidy in the development of HCC.<sup>62–64</sup> Gao et al, demonstrated the role of 16q loss in the downregulation of E-cadherin in human ovarian cancer cells (OV-5P) by isolating mesenchymal variants from clonal epithelial populations.<sup>65</sup> In addition, it

has been reported that trisomic epithelial cancer cells always demonstrate TWIST1-positive status in human colorectal cancer.<sup>66</sup> We also identified a few genes which are highly amplified in HCC tissue and have a great impact on the expression of E-cadherin. ARNT (13% amplification frequency in TCGA dataset) encodes the aryl hydrocarbon receptor nuclear translocator protein, which has been identified as the beta subunit of a heterodimeric transcription factor, hypoxia-inducible factor 1 (HIF1). The HIF1 $\alpha$  factor is reported to directly regulate TWIST and further alter E-cadherin expression<sup>67</sup> as well as that of Snail factor.<sup>68</sup> PBX1 encodes the Pre-B-cell leukemia transcription factor 1 (PBX1) and is found to occur at a high frequency of amplification in HCC (12% in TCGA dataset). Risolino et al, reported that PBX1 modulated TGF- $\beta$ -related E-cadherin expression by inducing SMAD3.<sup>69</sup> Besides PBX1, another gene demonstrating a





studies on whether Twist interacts with Snail/slugg protein and further affects E-cadherin signaling are contradictory. In chromatin immunoprecipitation (ChIP) assays, Twist1 was revealed to recruit Snail/slugg proteins through the interaction between the twist1 protein and the C-terminal zinc fingers of snail/slugg.<sup>81</sup> Twist1 is reported to downregulate snail mRNA expression in KYSE-30 cells.<sup>82</sup> However, Yang et al, failed to observe an induction of Snail in human mammary epithelial cells when Twist expression was altered, and they suggested that Twist and Snail may function independently in affecting E-cadherin expression.<sup>79</sup>

ZEB1 and its mammalian paralog ZEB2 both belong to the ZEB family within the zinc-finger class of homeodomain transcription factors. Among these, ZEB1 has been demonstrated to repress E-cadherin transcription by binding to two E-box sequences in its promoter region and interacting with the SWI/SNF family protein BRG1. It seems that the involvement of BRG1 is required for the regulation of ZEB1.<sup>83</sup> ZEB2 also represses the activity of the E-cadherin promoter by binding to a conserved E-box sequence.<sup>84</sup> However, ZEB2 plays a role in the TGF $\beta$  signaling pathway and is activated by full-length SMADs.<sup>85</sup> In addition, a previous study also reported that a complex of Cdc42 GTPase-activating protein (CdGAP) and ZEB2 mediated the repression of E-cadherin expression.<sup>86</sup>

## Epigenetic Mechanisms Play An Important Role In E-Cadherin Expression

Epigenetics is the study of heritable phenotype changes that do not involve alterations in the DNA sequence,<sup>87</sup> including four DNA modifications, 16 classes of histone modifications<sup>88</sup> and non-coding RNA (Figure 2). Recently, growing evidence suggests that epigenetic alterations play important roles in CDH1 expression and indeed lead to the development of HCC.<sup>89</sup>

## DNA Methylation In The Regulation Of CDH1 Expression

Typically, DNA methylation occurs at the 5'-position of the cytosine (5mC) of CpG dinucleotides catalyzed by DNMTs.<sup>90</sup> It generally serves as a down-regulator of target gene expression when a hypermethylation area exists at the promoter region.<sup>91</sup> Recent studies have revealed the aberrant 49% methylation of CDH1 in HCC using bisulfite genomic sequencing and ChIP assays.<sup>92,93</sup> Similarly,

a study focused on methylation status during multistep hepatocarcinogenesis also reported that the frequency of E-cadherin hypermethylation in premalignant lesions is less than 7%, whereas the frequency remarkably increased to 30% in HCC.<sup>94</sup> These findings suggest a significant driving role of CDH1 hypermethylation in the development of HCC. DNMT1, DNMT3A1 and DNMT3A2 are reported to selectively induce hypermethylation of the CDH1 promoter,<sup>95</sup> and the hypermethylation of CpG islands is highly associated with E-cadherin suppression at both the mRNA and protein levels.<sup>96</sup> Moreover, several studies have provided consistent evidence for the role of DNMT1 depletion in restoring E-cadherin expression via demethylation,<sup>97</sup> and increasing the methylation level of CpG islands around the promoter region can result in hepatocarcinogenesis through a reduction in E-cadherin expression.<sup>15,98</sup>

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are regarded as one of the most important risk factors in the progression of HCC. In China, most HCC cases are associated with HBV infection.<sup>99</sup> In HBxAg-positive cells, E-cadherin was found to be suppressed, and this phenomenon was associated with hypermethylation of the E-cadherin promoter.<sup>96</sup> This kind of E-cadherin suppression is dependent on DNMT1 because both the RNA and protein levels of DNMT1 were significantly increased in HBx-expressing cells, and the expression of E-cadherin was completely restored after treatment with a DNMT inhibitor (5'-Aza-2'dC).<sup>100</sup> In the study of HCV, 5'-Aza-2'dC can also reverse the E-cadherin suppressive function caused by the HCV core protein. The expression levels of both DNMT1 and DNMT 3B were dramatically increased in HCV core-expressing cells which lead to the suppression of E-cadherin.<sup>101</sup> However, the detailed mechanism through which the hepatitis virus interacts with methyltransferase remains obscure.

The transcription factor Snail is one of the most studied regulators of CDH1 DNA methylation in HCC.<sup>75</sup> Lim et al, clearly demonstrated that Snail plays a central role in CpG methylation of the E-cadherin promoter by recruiting DNMT1 to a specific site, which maintains DNA methylation and is implicated in tumorigenesis.<sup>102</sup> Furthermore, Snail is crucial in maintaining the aberrant methylation status of the CDH1 promoter in HCC, as DNMT1 cannot regulate the progression of methylation at the CDH1 promoter alone without snail participation.<sup>103</sup>

## Histone Methylation In The Regulation Of CDH1 Expression

Histone methylation typically takes place at arginine or lysine amino acid residues at the protein sequence. The former is controlled by protein arginine methyltransferases and the latter by protein lysine methyltransferases (PKMTs).<sup>104</sup> Histones package and histone methylation, typically at the tail of the H3 and H4 histones, will epigenetically influence gene expression because of the variable space structure.<sup>105,106</sup> Trimethylation of H3 lysine 4 (H3K4me3) and trimethylation of H3 lysine 36 (H3K36me3) are associated with active transcription, whereas trimethylation of H3 lysine 27 (H3K27me3), di- and trimethylation of H3 lysine 9 (H3K9me2/3) and trimethylation of H4 lysine 20 (H4K20me3) are associated with repressed gene expression.<sup>107,108</sup>

Interestingly, a web of interactions appears to tightly coordinate DNA and histone methylation in E-cadherin regulation networks. For example, a previous study reported that the expression levels of G9a, which is recruited by Snail, are positively correlated with the H3K9me2 levels of CDH1.<sup>109</sup> However, the relationship between H3K9me2 and E-cadherin expression is more complicated than once thought. In breast cancer, knockdown of G9a can restore E-cadherin expression by suppressing H3K9me2 and blocking DNA methylation. However, in HCC, E-cadherin expression is unchanged following knockdown of G9a. This contradiction may be explained by the involvement of other pathways, including TGF- $\beta$  signaling in HCC.<sup>110</sup> TGF- $\beta$  is reported to be a strong regulator of epigenetics. It can promote SNAI1 expression by removing histone H3 lysine trimethylation marks via the induction of lysine demethylase 6B (KDM6B) levels in breast cancer.<sup>111</sup> Feng et al, explored the epigenetic function of basil polysaccharide and demonstrated that it could suppress the expression of G9a, H3K9me2 and HIF-1 $\alpha$ , which regulated  $\beta$ -catenin<sup>112</sup> and twist,<sup>113</sup> and further increased the expression of E-cadherin in HCC. However, the causal relationship was not fully explored in their study.<sup>114</sup>

In addition, another histone methylation modification type, H3K27me3, is also reported to be correlated with EZH2 and E-cadherin expression. EZH2 is a PKMT and is capable of mono-, di-, and especially trimethylation of Lys-27 on histone 3 (H3K27).<sup>115</sup> A close relationship between H3K27me3 and EZH2 has been reported in HCC.<sup>116</sup> In a study of non-tumorigenic hepatocytes, EZH2 was demonstrated to directly interact with Snail with the

help of lncRNA HOTAIR.<sup>117</sup> Interestingly, Li et al, have discovered that CLDN14, as a novel direct target of EZH2-mediated H3K27me3, is involved in the Wnt/ $\beta$ -catenin pathway to regulate E-cadherin expression.<sup>118</sup>

## Histone Acetylation And Deacetylation Of CDH1 Itself And Other Factors

Typically, acetylation targets Lys residues in the amino-terminal tails of core histone proteins, which is catalyzed by histone acetyl transferases and histone deacetylases (HDACs). The acetylation of histones changes histone positive charge, mainly at the lysine position. Therefore, highly acetylated histones can lead to more accessible chromatin and this is associated with active transcription.<sup>119</sup> In HCC, many studies have demonstrated that HDAC1, which is highly expressed in HCC, is essential for different kinds of classical pathway to change the transcription of CDH1. Lei et al,<sup>120</sup> reported that HDAC1 might downregulate the promoter activity of E-cadherin in regulating the TGF- $\beta$  pathway. However, because of the complexity of the TGF- $\beta$  pathway, the specific target of HDAC1 and the underlying mechanism remain unknown. It is currently thought that HDAC1 interacts with snail and further regulates E-cadherin expression. In a pancreatic cancer study, silencing of E-cadherin was mediated by a transcriptional repressor complex containing Snail and recruited HDAC1/2.<sup>99</sup> Early studies demonstrated that DNMT and HDAC appear to act as synergistic regulators in silencing target genes.<sup>121,122</sup> Snail recruits related enzymes which may cooperate in the regulation progression. Besides snail protein, it has also been found that ZEB1 can bind to its target gene (including CDH1) and induce a decrease in histone H3K27 acetylation.<sup>123</sup> In addition, HDAC1 is reported to directly regulate the H3K9Ac and H4K16Ac levels of the CDH1 promoter to downregulate the expression of E-cadherin through the ten-eleven-translocation (TET) family protein TET2.<sup>124</sup>

Xu et al, observed that histone deacetylase inhibitors could influence the histone stability of the CDH1 gene and upregulate SNAI1 expression. The above-mentioned inhibitors can upregulate the expression of COP9 signalosome 2 (CSN2) to combine with Snail and exposes its acetylation site. As a result, the hyperacetylation of Snail represses its degradation and downregulates the expression of E-cadherin.<sup>125,126</sup> The modification of snail has received increasing attention in recent research. P300, which belongs to a coactivator family together with

CREB-binding protein, contains a histone acetyltransferase (PAT/HAT) domain, and is newly identified that it could acetylate Snail and further lead to Snail transcriptional repression.<sup>127</sup> In HCC, knockdown of p300 expression can also recover E-cadherin expression.<sup>128</sup> Furthermore, the expression of Twist was also decreased by the suppressive binding of H3K27ac to the Twist promoter, which is induced by inhibition of the PI3K/Akt and Wnt/ $\beta$ -catenin signaling pathways.<sup>129</sup>

## lncRNAs Function In E-Cadherin Signaling Through Several Crucial Pathways And Factors

Non-coding RNAs (ncRNA) are human genes that do not code for proteins. ncRNAs are divided into two groups: small ncRNAs (<200 nt, including miRNAs, siRNAs, piRNAs) and long ncRNAs (longer than 200 nt). The latter function as various types of gene expression regulator, including functioning as molecular signals, molecular decoys (by binding and titrating away a protein or RNA target to negatively regulate expression), protein guides (which direct the localization of specific proteins to their target to form a complex) and scaffolds (to bind relevant molecular components).<sup>130</sup> Recently, it has been revealed that the dysregulation of several lncRNAs can affect the expression of E-cadherin in the pathogenesis of HCC (Table 1), with the involvement of some important signal transduction pathways. Here, we have summarized the lncRNAs involved in E-cadherin signaling.

lncRNAs can regulate E-cadherin expression by affecting the Wnt/ $\beta$ -catenin pathway. lncRNA ATB<sup>131</sup> and lncRNA H19<sup>132</sup> were found to be regulated by TGF- $\beta$  signaling and further function as oncogenic regulators to decrease the expression of E-cadherin in HCC. lncRNA ATB downregulates E-cadherin expression by sharing the response elements of miR-200 with ZEB1 and ZEB2,<sup>131</sup> whereas lncRNA H19 activates the CDC43/PAK1 signaling pathway by targeting miR-15b.<sup>133</sup> Activation of the Wnt/ $\beta$ -catenin pathway has been identified to affect E-cadherin expression. Ge et al, demonstrated that lncRNA HOTAIR, a HOX transcript antisense intergenic RNA, could activate the Wnt pathway in esophageal squamous cell carcinoma cells by inhibiting WIF-1 expression.<sup>134</sup> In HCC, lncRNA HOTAIR was found to be recruited by the Snail protein, and mediated the Snail/EZH2 interaction, as mentioned above.<sup>117</sup> Recently, lncRNA-NEF was reported to upregulate E-cadherin expression through the suppression of Wnt/

$\beta$ -catenin.<sup>135</sup> Similarly, lncRNA SNHG5 decoys miR-26-5p to activate the Wnt/ $\beta$ -catenin pathway and downregulate E-cadherin expression.<sup>136</sup> Researchers have also reported that lncRNA OGFRP21<sup>137</sup> and lncRNA CRANDE<sup>138</sup> can influence E-cadherin expression through the Wnt/ $\beta$ -catenin pathway in HCC, but the specific mechanisms underlying these phenomena remain to be explored.

Certain lncRNAs can significantly affect E-cadherin expression by directly targeting important transcription factors in E-cadherin signaling. ZEB1 and ZEB2, zinc-finger and homeodomain proteins are important components of a network of transcription factors that control E-cadherin expression. Several lncRNAs, including ATB,<sup>131</sup> HULC,<sup>139</sup> HOTAIR,<sup>140</sup> CARLo-5<sup>141</sup> and ZEB-AS1,<sup>142</sup> are reported to target ZEB1 and ZEB2 and interact with paired E-box-like promoter elements to control transcriptional activity.<sup>143</sup> In addition, researchers have found that certain lncRNAs function via E-box repressors such as snail, slug and twist1, to downregulate the expression of E-cadherin. The AMPK-associated lncRNA MITA1 was reported to upregulate slug expression.<sup>144</sup> lncRNA UBE2CP3 and lncRNA UCA1 can suppress the expression of E-cadherin by upregulating Snail expression,<sup>145,146</sup> while lncRNA Sox2ot,<sup>147</sup> lncRNA CARLo-5<sup>141</sup> and SPRY4-IT1<sup>148</sup> can suppress the expression of E-cadherin by activating Twist.

## MicroRNAs Can Demonstrate A Suppressive Or Oncogenetic Role In HCC By Affecting E-Cadherin Expression

As mentioned above, microRNAs (abbreviated to miRNAs) are a large ncRNA family. In a difference from lncRNAs, miRNAs function via base-pairing with complementary sequences (mostly in the 3'-UTR) within mRNA molecules to silence mRNA transcription.<sup>149</sup> Altered expression levels of various types of miRNA have been observed in human cancers, especially in HCC.<sup>150</sup>

miR-122 is reported to play a critical role in liver diseases such as steatohepatitis, fibrosis and HCC.<sup>151</sup> Hsu et al, reported that miR-122 regulates polyploidization in murine liver, which is thought to be a key step in the progression of HCC.<sup>152</sup> Jin et al, reported that miR-122 may affect the expression of E-cadherin by regulating the snail and Wnt/ $\beta$ -catenin pathway.<sup>153,154</sup> Conversely, Liang et al, demonstrated that miR-122 can be sequestered by HBx-LINE1, a hybrid RNA transcript of human LINE1



**Table 1** The lncRNA And microRNA Involved In E-Cadherin Expression In HCC

Molecules	Expression In HCC	Regulation Of CDH1	Relevance To HCC	Refs.
<b>LncRNA</b>				
NEF	Down	Positive	FOXA2—LncRNA-NEF— $\beta$ -catenin—E-cadherin	135
MITA1	Up	Negative	AMPK or DNA methylation—MITA1—Slug—E-cadherin	144
ATB	Up	Negative	TGF- $\beta$ activity—LncRNA-ATB—combinemiR-200—ZEB1 ZEB2—E-cadherin	131
H19	Up	Negative	TGF- $\beta$ —Sox2—H19—miR-15b—CDC42/PAK1—E-cadherin	133,166
HULC	Up	Negative	HULC decoy—miR-200a-3p—ZEB1—E-cadherin	139
HOTAIR	Up	Negative	HOTAIR—miR-23b-3p—ZEB1—E-cadherin	117,140
COX2	Up	Negative	COX2—E-cadherin	167
p21	Down	Positive	LncRNA-p21—decoysmiRNA-9—E-cadherin	168
UBE2CP3	Up	Negative	LncRNAUBE2CP3—miR-138-5pN-cadherin, Snail—E-cadherin	145
Sox2ot	Up	Negative	LncRNASox2ot—Twist1, N-cadherin—E-cadherin	147
SNHG20	Up	Negative	LncRNASNHG20—combineEZH2—E-cadherin	169
SNHG5	Up	Negative	LncRNASNHG5decoymiR-26a-5p—GSK3 $\beta$ —Wnt/ $\beta$ —E-cadherin	170
OGFRP1	Up	Negative	LncRNAOGFRP1—AKT/mTOR, Wnt/B-catenin—E-cadherin	137
SPRY4-IT1	Up	Negative	LncRNASPRY4-IT1—Twist1, Vimentin—E-cadherin Or, SPRY4-IT1—combineEZH2—E-cadherin	148
PVY1	Up	Negative	LncRNAPVT1—guideEZH2—miR-214Or, PVT1—E-cadherin	171
CARLo-5	Up	Negative	LncRNA—Twist1, ZEB1—E-cadherin	141
UCA1	Up	Negative	LncRNA—miR-203—Snail2—E-cadherin	146
Linc00152	Up	Negative	Linc00152—combineEZH2—E-cadherin	172
BANCER	Up	Negative	LncRNA BANCER—E-cadherin	173
CCAT2	Up	Negative	LncRNACCAT2—snail—E-cadherin	174
DQ786243(LncDQ)	Up	Negative	LncDQ—EZH2—combineH3K27me3—E-cadherin	175
ZEB1-AS1	Up	Negative	LncRNAZEB1-AS1—E-cadherin	142
HOST2	Up	Negative	LncRNAHOST2—JAK2-STAT3/or Snail, Slug, Twist-E-cadherin	176
Linc00857	Up	Negative	LINC00857—E-cadherin	177
CRNDE	Up	Negative	CRANDE—Wnt/ $\beta$ -catenin—E-cadherin	138
NRON	Down	Positive	LncRNA NRON—snail, catenin, E-cadherin	178
AK021443	Up	Negative	AK021443—E-cadherin,Snail	179
<b>Microrna</b>				
Mir-199-3p	Down	Positive	199-3p—Notch1—E-cadherin	180
Mir-186	Up	Negative	RUNX3combinemir-186promotorRunx3—mir-186—E-cadherin	181
Mir-122	Down	Positive	Mir-122—snail/Wnt/b—E-cadherin	152-154
Mir-9	Up	Negative	Mir-9—(direct)E-cadherin	182,183
Mir-29a	Down	Positive	mir-29a—DNMT—CpG methylation—E-cadherin	184
Mir-26a-5p	Down	Positive	Mir-26a-5p—E-cadherin	185
Mir-33a	Down	Positive	Hypoxia, HIFs—mir-33a—twist1—E-cadherin	186
Mir-133b	Down	Positive	Mir-133b—sirt1—GPC3—Wnt/b-catenin—E-cadherin	187
Mir-451	Down	Positive	Mir-451—c-Myc—Erk1/2; or, c-Myc—(GSK-3b?)—snail—E-cadherin	188
Mir-153	Down	Positive	Mir-153—snail 3'UTR—E-cadherin	189
Mir-130a-3p	Down	Positive	Mir-130a-3p—Smad4—E-cadherin	190
Mir-130b	Up	Negative	Mir-130b—PPAR—E-cadherin	191
Mir-192	Down	Positive	Mir-192—SLC39A6—snail—E-cadherin	192
Mir-876-5p	Down	Positive	Mir-876-5p—BCORLI—E-cadherin	193
Mir-200a/b	Down	Positive	Mir-200—ZEB1/2—E-cadherin	156,157,194,195
Mir-30c/203a	Down	Positive	HCV core protein—mir-30c/203a—E-cadherin	196
Mir-16	Down	Positive	Mir-16—PI3K/Akt—E-cadherin	197

(Continued)

Table I (Continued).

Molecules	Expression In HCC	Regulation Of CDH1	Relevance To HCC	Refs.
Mir-26a	Down	Positive	Mir-26a—EZH2mRNA—EZH2—E-cadherin	198
Mir-655-3p	Down	Positive	655-3p—ADAM10—E-cadherin	158
Mir-185	Down	Positive	Mir-185—six2—E-cadherin	199
Mir-31	Down	Positive	Mir-31—HDAC2—E-cadherin	200
Mir-155	Down	Positive	TGF- $\beta$ —mir-155—E-cadherin	201
Mir-26b	Down	Positive	Mir-26b—USP9X—Smad4orTGF- $\beta$ —E-cadherin	202
Mir-148b	Down	Positive	Mir-148b—Wnt/b—E-cadherin	203
Mir-148a	Down	Positive	Mir-148a—Met/Snail—E-cadherin	204
Mir-30a-3p	Down	Positive	Mir-30a-3p—E-cadherin	205
Mir-103	Up	Negative	Mir-103—LATS2(Hippo)—E-cadherin	206
Mir-101	Down	Positive	Mir-101—ZEB1—E-cadherin	207
Mir-491	Down	Positive	Mir-491—E-cadherin	208
Mir-141-3p	Down	Positive	Mir-141-3p—GP73target—E-cadherin	209
Mir-214-5p	Down	Positive	Mir-214-5p—WASL target—E-cadherin	159
Mir-152	Down	Positive	Mir-152—DNMT1—methylation—E-cadherin	160
Mir-564	Down	Positive	Mir-564—targetGRB2-ERK1/2-AKT—E-cadherin	210

and the HBV-encoded X gene generated in tumor cells in HBV-positive HCC, to down-regulate the expression of E-cadherin.<sup>155</sup>

Various miRNAs demonstrate an oncogenic role by altering different pathways. However, there is increasing evidence to suggest that miRNAs play critical roles as tumor suppressors. miR-200 has been identified as a marker for E-cadherin-positive cancer cells. Sun-Mi Park et al, have reported that miR-200 directly targeted ZEB1 and ZEB2 to upregulate E-cadherin in 60 cell lines in a drug screening panel of human cancer cell lines at the National Cancer Institute (NCI60).<sup>156</sup> In HCC, Ding et al, proposed the miR-200b could regulate E-cadherin signaling by ZEB1.<sup>157</sup> Wu et al, observed that miR-655-3p was significantly down-regulated in HCC tissues and HCC cell lines. They reported that miR-655-3p directly targets a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), which indirectly regulates the  $\beta$ -catenin signaling pathway and upregulates E-cadherin protein levels during HCC progression.<sup>158</sup> Li et al, discovered that miR-214-5p targets Wiskott-Aldrich Syndrome Like (WASL) protein and indirectly upregulates the expression of E-cadherin. Restoring WASL expression could reverse the inhibition effect of miR-214-5p on E-cadherin.<sup>159</sup> Additionally, Huang et al,<sup>160</sup> have reported an epigenetic function for miRNA-152 as a tumor suppressor. They found that miR-152 levels are frequently correlated with DNMT1 mRNA expression in HBV-related HCCs, and

DNMT1 can inhibit the hypermethylation of E-cadherin to increase E-cadherin expression in HCC.

## E-Cadherin Is A Promising Indicator And Target For The Treatment Of HCC

In view of the aberrant E-cadherin expression that is associated with the initiation and progression of HCC, E-cadherin may serve as a promising prognostic indicator. A study of the expression levels of E-cadherin in 41 HCC samples suggested that the survival rate in patients with negative expression of E-cadherin was significantly lower.<sup>161</sup> A meta-analysis of 2439 patients in 30 studies also demonstrated the predictive potential of E-cadherin for the prognosis of HCC patients.<sup>162</sup> Epigenetic drugs represent an attractive therapeutic strategy. Panobinostat, a novel pan-HDAC-inhibitor has demonstrated substantial efficacy in several preclinical models of cancer, and is reported to upregulate CDH1 and demonstrated the highest preclinical efficacy when combined with sorafenib in HCC models.<sup>163</sup> Apart from epigenetic drugs, gene therapy approaches have gained more attention. The miRNAs mentioned above could potentially serve as a component of E-cadherin gene therapy because of their specificity and ease of handling. Therefore, the gene of interest in the form of a tumor suppressor short-interfering RNA can be efficiently delivered to cells using viruses such as lentiviruses, adenoviruses or adeno-associated viruses.<sup>164</sup>

However, viral carriers can cause severe side effects, which might limit the application of viral vectors in future clinical practice. Therefore, other non-viral-based transgene vectors<sup>165</sup> are worthy of exploration.

## Conclusion

Previous research in different cancer types has revealed the basic regulatory framework for E-cadherin signaling. Patients with HCC tend to have a high-risk of multi-centric tumor occurrence and E-cadherin is a crucial factor in this process. Here, we present an overview of E-cadherin regulatory mechanisms, focusing on genetic and epigenetic alterations. Based in part on the data from the TCGA dataset, we can find some highly altered genes associated with E-cadherin which are poorly explored in HCC. An exhaustive exploration of the epigenetic regulatory mechanisms of E-cadherin, including DNA methylation, histone modifications and ncRNA, will help us to fully understand the special biological processes involved in HCC initiation and metastasis.

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## Disclosure

The authors report no conflicts of interest in this work.

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