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CLINICAL & BASIC RESEARCH

Expression of DKK1 in Endometrial Endometrioid Carcinoma and Its Correlation with Wnt/β-catenin Signalling Pathway

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abstract: ***Objectives:*** Endometrial cancer is the most prevalent form of cancer affecting female reproductive organs. The most common histologic type, endometrioid carcinoma, accounts for 75–80% of all endometrial cancer cases. Studies on DKK1 expression profiles and their inhibitory role in the Wnt signalling pathway in the genesis and development of endometrial carcinoma are scarce. This study aimed to investigate DKK1 expression in endometrial carcinoma and its correlation with the Wnt/β-catenin pathway. ***Methods:*** A total of 160 formalin-fixed paraffin-embedded samples were included in this study (50 cases of endometrial atypical hyperplasia, 50 cases of endometrioid endometrial carcinoma, 30 cases of proliferative endometrium and 30 cases of secretory endometrium). The expression patterns of DKK1, E-cadherin, β-catenin and c-Myc in endometrial atypical hyperplasia and carcinoma were investigated and compared with that of proliferative and secretory endometrium. Immunohistochemistry and analysis were performed from July 2018 to June 2020. ***Results:*** A decreasing pattern of immunopositivity for DKK1, E-cadherin and β-catenin from proliferative/secretory endometrium to endometrial atypical hyperplasia and endometrioid carcinoma was found. Increasing c-Myc immunopositivity was noted from proliferative/secretory endometrium to endometrial atypical hyperplasia and endometrioid carcinoma. Moreover, decreasing DKK1 immunopositivity was well correlated with E-cadherin, β-catenin and c-Myc immunopositivity. ***Conclusion:*** Decreasing DKK1 positivity from benign endometrium to endometrioid carcinoma suggests a negative regulatory function of DKK1 in endometrioid carcinoma. DKK1 is downregulated in the Wnt signalling pathway in endometrioid endometrial carcinoma. Therefore, DKK1 is promising as a biomarker for screening endometrioid carcinoma. Future studies should examine the reactivation of the DKK1 gene, which may be a valuable strategy for antagonising the Wnt signalling pathway.

Keywords: Endometrioid Carcinoma; DKK1; Wnt/β-catenin pathway; β-catenin; E-cadherin; India.

Advances in Knowledge

- *DKK1 shows a decreasing trend of immunoexpression from the benign phase of the endometrium to endometrioid endometrial carcinoma.*
- *Expression of DKK1 is well correlated with the markers of the Wnt signalling pathway (β-catenin, E-cadherin and c-Myc).*
- *DKK1 antagonises the Wnt signalling pathway.*

Application to Patient Care

- *DKK1 can be a promising biomarker in screening the progression of endometrioid endometrial carcinoma.*
- *Reactivation of the DKK1 gene can be a valuable strategy for antagonising the Wnt signalling pathway in endometrioid endometrial carcinoma.*

Endometrial cancer is the most prevalent invasive gynaecologic malignancy among American women, accounting for 7% of the estimated new cancer cases in 2021.[1] Incidence and death rates of endometrial cancer have been increasing by an average of 1.1% and 0.3% per year, respectively.[2] The most common histological type, endometrioid adenocarcinoma, constitutes 75–80% of endometrial cancers. The disease mostly affects postmenopausal women, with an average age of 60 years at diagnosis, while only 5% of the cases are reported in women younger than 40 years of age.[3] In India, endometrial cancer ranks third among female genital tract malignancies, after carcinoma cervix and carcinoma ovary.[4] A majority of endometrial cancer cases are diagnosed in the early stages because of abnormal uterine bleeding. The best diagnostic strategy for determining endometrial cancer in postmenopausal patients presenting with abnormal uterine bleeding remains controversial. Nowadays, endometrial biopsy and hysteroscopy have almost replaced dilatation and curettage for the diagnosis and management of endometrioid carcinoma.[5] Recent studies have demonstrated that the first step in the diagnostic pathway should be the measurement of endometrial thickness, followed by endometrial

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sampling.[6] Clinical assessment, radiological evaluation and histopathological examination have led the way to the study of molecular pathways such as the Wnt signalling pathway. The Wnt signal transduction pathway is activated by the binding of a Wnt protein to a cell surface receptor. E-cadherin (a cell adhesion molecule that forms adherens junctions between cells), β-catenin (a subunit of the cadherin protein complex) and c-Myc (a transcription factor protein that regulates cell proliferation) are integral components of the Wnt signalling pathway. Abnormalities in the Wnt signalling transduction pathway are responsible for the genesis and development of certain human malignant tumours [Figure 1].[7]

Attempts have been made to assess various regulators in the Wnt signalling pathway as targets for the diagnosis and treatment of malignant tumours. Several candidate markers, such as E-cadherin, β-catenin and c-Myc, have been proposed for cytologic or histologic samples in endometrial carcinoma.[8] As a negative regulator in the Wnt signalling pathway, DKK1 can inhibit Wnt activation during tumour progression.[9,10] Earlier studies focusing on the colorectum and placenta have highlighted that DKK1 is prominently expressed in normal cells but absent in cancer cells.[11] At present, studies on DKK1 expression profiles in endometrial carcinoma are scarce.[12] DKK1 expression pattern in endometrial carcinoma and its correlation with other components of the Wnt pathway, especially β-catenin, E-cadherin and c-Myc, have not been studied so far in India. This study will investigate the expression patterns of DKK1, E-cadherin, β-catenin and c-Myc in endometrial carcinoma. Moreover, the expression patterns of these markers in endometrial atypical hyperplasia and carcinoma will be compared with that of proliferative and secretory endometrium.

Methods

This retrospective study was conducted at the Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, India. It included formalin-fixed paraffin-embedded samples of endometrial lesions from patients between the ages of 21 to 77 years which were collected between January 2005 and March 2018. Endometrial samples from younger patients were primarily taken to exclude the causes of infertility and abnormal uterine bleeding. Standard morphological criteria were employed for the diagnosis and selection of cases and control groups. One section of each sample was stained with haematoxylin and eosin (H&E), and four-step sections on coated slides were utilised for DKK1, E-cadherin, β-catenin and c-Myc immunohistochemistry (IHC). IHC and data analysis were performed from July 2018 to June 2020.

IHC was performed for DKK1, E-cadherin, β-catenin and c-Myc using available monoclonal antibodies (DKK1, Abcam, 1:100; β-catenin, Thermo Scientific, 1:400; c-Myc, Thermo Scientific, 1:100; and E-cadherin, Thermo Scientific, 1:200). Serial 4-micron thick sections were cut from the selected representative paraffin-embedded tissue blocks and 3-aminopropyl triethoxysilane-coated slides were employed for IHC. The slides were deparaffinised, followed by rehydration to decrease the concentration of alcohol. For DKK1, E-cadherin and c-Myc immunostains, antigen retrieval was performed by heating the sections in a citrate buffer inside a 600-watt microwave oven at full power for 30 minutes. For β-catenin, Tris-ethylenediaminetetraacetic acid buffer at pH 8 was used for heat-mediated antigen retrieval. To diminish nonspecific immunostaining (i.e. endogenous peroxidase activity), each slide was treated with methanol containing 4% hydrogen peroxide for 30 minutes. For all immunostains, the sections were then overlaid with an adequate amount of appropriately diluted primary antibody, followed by overnight incubation at 4°C in a humid chamber. After three changes of washing (5 minutes each) in Tris-HCl buffer peroxidase, conjugated streptavidin was applied to cover the sections, followed by incubation at room temperature for 30 minutes. Each section was subsequently covered with substrate chromogen solution freshly prepared by dissolving 50 µl of diaminobenzidine (DAB) chromogen into 1 ml of DAB substrate buffer. The sections were counterstained with haematoxylin for 10 seconds, followed by mounting with DPX. During the staining of each batch, appropriate positive and negative controls (by omitting the primary antibody) were employed.

IHC stains (DKK1, cytoplasmic; β-catenin, membranous; c-Myc, cytoplasmic and nuclear; E-caderin, membranous) were reviewed and analysed in conjunction with H&E stained slides. The immunoreactive score (IRS) was obtained by multiplying the intensity score (0 = no staining; 1 = weak; 2 = moderate; and 3 = strong staining) and percentage score (0: nil; 1:<10%; 2: 10–50%; 3: 51–80%; and 4:>80%). Thus, the total IRS score ranged from 0 to 12.[13] Two independent observers analysed the expression pattern of all four markers, following which an average was calculated for the final analysis. Appropriate statistical tests, including independent sample t-test, Chi-squared test and Pearson correlation test, were utilised to analyse the significance of the results between the cases and the

control groups using the Statistical Package for Social Sciences (SPSS), Version 21.0 (IBM Corp., Armonk, New York, USA) software programme. P <0.05 was considered statistically significant.

The study was approved by the Institutional Ethics Committee at AIIMS, New Delhi, India.

Results

This retrospective study evaluated a total number of 160 cases/samples, comprising 50 cases of endometrial atypical hyperplasia, 50 cases of endometrioid endometrial carcinoma, 30 cases of proliferative endometrium and 30 cases of secretory endometrium. Immunoprofiles using DKK1, E-cadherin, c-Myc and β-catenin were compiled, compared and analysed for different expression patterns in various groups of endometrium.

The age pattern of the proliferative group versus the secretory group was statistically insignificant (P = 1.000), while the age patterns of proliferative endometrium versus endometrial atypical hyperplasia; proliferative endometrium versus endometrial carcinoma; secretory endometrium versus endometrial atypical hyperplasia; secretory endometrium versus endometrial carcinoma; and endometrial atypical hyperplasia versus endometrial carcinoma were statistically significant (P <0.001).

DKK1 primarily exhibited cytoplasmic expression in the glandular epithelium during the proliferative phase and in endometrial atypical hyperplasia and endometrioid carcinoma. However, two cases of proliferative endometrium showed nonspecific nuclear positivity both in the glandular epithelium and the stroma. Secretory endometrium exhibited cytoplasmic immunopositivity both in the glandular and stromal cells. Squamous morules associated with endometrioid carcinoma also showed similar cytoplasmic immunopositivity. The cytoplasmic expression among the groups was studied. DKK1 immunopositivity of proliferative endometrium versus secretory endometrium was statistically insignificant (P = 0.183). Increased DKK1 immunopositivity was observed in proliferative endometrium compared with endometrial atypical hyperplasia and endometrioid carcinoma, which was statistically significant (P <0.001). DKK1 immunopositivity of endometrial atypical hyperplasia versus endometrioid carcinoma was statistically insignificant (P = 1.000). Secretory endometrium showed increased DKK1 immunopositivity as compared with endometrial atypical hyperplasia and endometrioid carcinoma, and the difference was statistically significant (P <0.001). DKK1 exhibited a decreasing trend of expression from endometrial atypical hyperplasia to grade I endometrioid carcinoma and finally to grade II endometrioid carcinoma. When individual grades were separately compared, the difference between endometrial atypical hyperplasia and grade I endometrioid carcinoma was statistically insignificant (P = 1.000); however, the difference was statistically significant between endometrial atypical hyperplasia and grade II endometrioid carcinoma (P = 0.048) [Figure 2].

E-cadherin exhibited membranous immunopositivity. E-cadherin immunopositivity of proliferative

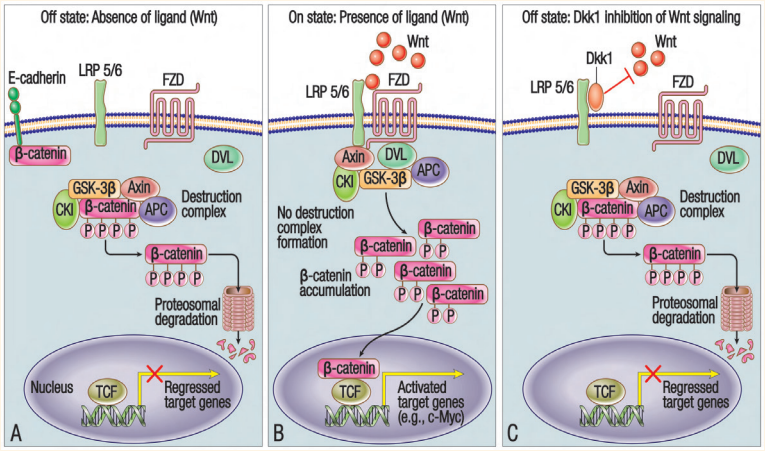

Figure 1: A: Absence of signalling molecule, that is, ligand (Wnt molecule), leads to the formation of 'destruction complex,' which, in turn, creates a hyperphosphorylated β-catenin destined for proteosomal degradation. Also depicted is E-cadherin binding to β-catenin and forming adherens junction. **B:** Wnt molecule binding to Frizzled (FZD)/LRP 5/6 receptors inactivates the 'destruction complex' and stabilises the hypophosphorylated β-catenin that then enters the nucleus to interact with the TCF/LEF family proteins to activate gene transcription. **C:** DKK1 binds to LRP5/6 co-receptor and blocks Wnt binding, which ultimately results in β-catenin degradation and the repression of gene transcription (Illustration was created by the authors).

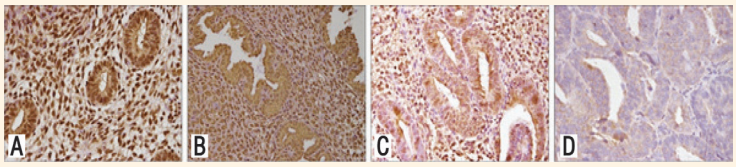

Figure 2: DKK1 immunopositivity at ×400 magnification of (**A**) proliferative endometrium, (**B**) secretory endometrium, (**C**) endometrial atypical hyperplasia and (**D**) endometrioid carcinoma.

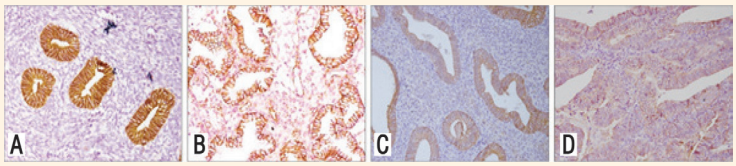

Figure 3: E-cadherin immunopositivity at ×400 magnification of (**A**) proliferative endometrium, (**B**) secretory endometrium, (**C**) endometrial atypical hyperplasia and (**D**) endometrioid carcinoma.

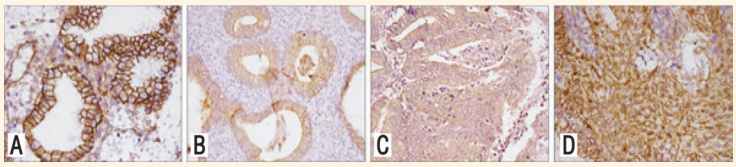

Figure 4: β-catenin immunopositivity of (**A**) secretory endometrium at ×400 magnification, (**B**) endometrial atypical hyperplasia at ×100 magnification, (**C**) endometrioid carcinoma at ×400 magnification and (**D**) nuclear positivity in endometrioid carcinoma at ×400 magnification.

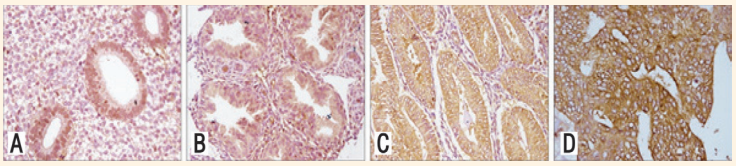

Figure 5: c-Myc immunopositivity of (**A**) proliferative endometrium at ×100 magnification, (**B**) secretory endometrium at ×200 magnification, (**C**) endometrial atypical hyperplasia at ×200 magnification and (**D**) endometrioid carcinoma at ×400 magnification.

endometrium versus secretory endometrium was statistically insignificant ($P = 1.000$). Immunopositivity of both proliferative endometrium and secretory endometrium were higher than those of endometrial atypical hyperplasia and endometrioid carcinoma, and the difference in immunopositivity among them was statistically significant ($P < 0.001$). A statistically significant difference was also observed between endometrial atypical hyperplasia and endometrioid carcinoma ($P < 0.001$) [Figure 3].

Membranous β-catenin expression was studied among the groups. Nuclear β-catenin was observed in 14% (n = 7/50) of the endometrioid carcinomas, excluding the areas of squamous morule formation, which also showed nuclear positivity. β-catenin immunopositivity of proliferative endometrium versus secretory endometrium was statistically insignificant ($P = 1.000$). In this study, both proliferative endometrium and secretory endometrium showed increased immunopositivity of β-catenin compared with endometrial atypical hyperplasia and endometrioid carcinoma; the difference in β-catenin immunopositivity among them was statistically significant ($P < 0.001$). Immunopositivity in endometrial atypical hyperplasia was also statistically significant ($P < 0.001$) when compared with that of endometrial carcinoma [Figure 4].

Cytoplasmic c-Myc immunopositivity among the groups was evaluated. Additionally, nuclear expression was noted in 14 and four cases of proliferative and secretory endometrium, respectively. When c-Myc immunopositivity of proliferative endometrium was compared with that of secretory endometrium, the difference was statistically insignificant ($P = 1.000$). Increased immunopositivity was observed in endometrioid carcinoma compared with proliferative endometrium and endometrial atypical hyperplasia, and the difference in c-Myc immunopositivity among

Table 1: Comparison of immunopositivity between grade I and grade II endometrioid carcinoma

IHC and grade*	n	Mean IRS ± SD	*P* value
DKK1			0.207
1	39	4.10 ± 2.222	
2	11	3.18 ± 1.601	
E-cadherin			0.853
1	39	2.92 ± 1.645	
2	11	2.82 ± 1.662	
β-catenin			0.492
1	39	3.31 ± 1.360	
2	11	3.64 ± 1.502	
c-Myc			0.716
1	39	8.67 ± 3.198	
2	11	8.27 ± 2.970	

IHC = immunohistochemistry; IRS = immunoreactive score; SD = standard deviation.

**1 = Grade 1 and 2 = Grade 2.*

them were statistically significant ($P = 0.043$ and <0.001, respectively). By contrast, c-Myc immunopositivity of secretory endometrium versus endometrial atypical hyperplasia was statistically insignificant ($P = 0.384$), while c-Myc immunopositivity of secretory endometrium versus endometrioid carcinoma was statistically significant ($P < 0.001$) [Figure 5].

In the endometrial atypical hyperplasia group, statistically significant correlations between DKK1 and β-catenin immunopositivity as well as between E-cadherin and c-Myc immunopositivity were found. The remaining three groups did not show any significant correlation with the four IHC markers. The IHC comparison between the two age groups in endometrial atypical hyperplasia and endometrioid carcinoma, as well as between grade I and grade II endometrioid carcinomas, did not reveal any significant difference [Table 1].

Discussion

Endometrial cancer has surpassed cervical cancer as the most common gynaecologic malignancy. In the past few decades, cervical cancer was much more prevalent than endometrial cancer; however, earlier detection and eradication of cervical precursor lesions has reversed this scenario.[14] Endometrial carcinoma frequently occurs in peri- and post-menopausal women, with endometrioid carcinoma being the most common histological subtype.[3,15] PTEN genetic mutation occurs most frequently (39–83%) in endometrioid cancer; however, β-catenin mutation accounts for 31–47% of the cases.[16] β-catenin is an integral component of the Wnt signalling pathway, which is dysregulated in many human cancers. On the contrary, a negative regulator of the β-catenin pathway, DKK1, prevents tumour progression by inhibiting this signalling pathway.[9] Some studies have described the role played by DKK1 in non-endometrial tissues in both normal and corresponding malignant cells; however, studies on endometrial cancer are few in the extant literature in English.[11,12] Hence, the expression pattern of DKK1 in various groups of benign, atypical and malignant endometrium was evaluated and correlated with other markers, such as E-cadherin, β-catenin and c-Myc, of the Wnt pathway to demonstrate the relation among the groups [Figure 1].

DKK1 is a glycoprotein and a member of the Dkk family (Dkks) that is secreted by various cells throughout the human body.[17] The human *DKK1* gene maps to chromosome 10q11.2, which encodes a protein that acts as an antagonist in the Wnt signalling pathway by binding to and inhibiting LRP 5/6 [Figure 1C].[18]

Yi *et al*. demonstrated DKK1 positivity in both benign endometrium and endometrial carcinoma, where DKK1 was primarily distributed in the cytoplasm of glandular epithelium. They documented the 'high expression' of DKK1 predominantly in benign endometrium in contrast to the 'low expression' in endometrial cancer, suggesting that this reduction expression can be attributed to its negative regulatory function in the Wnt signalling pathway.[12] Decreasing DKK1 immunopositivity from proliferative or secretory endometrium to endometrial atypical hyperplasia and endometrioid carcinoma was also found. In the current study, DKK1 positivity was predominantly found in the cytoplasm of the glandular epithelium; however, stromal cells also showed weak cytoplasmic immunopositivity [Figure 2].

A significant difference in DKK1 immunopositivity between endometrial atypical hyperplasia and proliferative/secretory endometrium, as well as between endometrioid carcinoma and proliferative or secretory endometrium was also found. Though increased DKK1 immunopositivity was observed in endometrial atypical hyperplasia as compared with endometrioid carcinoma, it did not achieve statistical significance. Some studies have demonstrated reduced expression of β-catenin following treatment with exogenous DKK1, probably indicating that increased DKK1 binding to LRP5/6 inhibits Wnt signalling, which leads to the degradation of β-catenin.[19] Decreasing DKK1 positivity from benign endometrium to endometrioid carcinoma in the current study may suggest that the negative regulatory function of DKK1

is reduced from benign to malignant endometrium. Thus, at least in part, by inducing abnormalities in the Wnt signalling pathway, DKK1 plays a role in the genesis and development of endometrial carcinoma. Similar patterns of DKK1 alterations have also been reported in some other tumours, including colorectal cancer, placental choriocarcinoma and non-small cell lung cancers, where *DKK* genes were frequently silenced.[11,20] In the current study, decreasing positivity of DKK1 from proliferative or secretory endometrium to endometrial atypical hyperplasia and endometrioid carcinoma suggests the involvement of DKK1 in the early phase of endometrioid carcinoma through the suppression of the Wnt pathway.

Cell surface glycoprotein, E-cadherin, which has a molecular weight of 120 kDa, is a major cadherin molecule expressed by epithelial cells. It binds to catenin to form a cadherin-catenin complex that plays a critical role in intercellular adhesion [Figure 1A].[21]

Shih *et al*. demonstrated that the cytoplasmic expression of E-cadherin in endometrial glandular cells occurred primarily in the proliferative phase and decreased in the secretory phase.[8] In contrast to their study, strong membranous immunopositivity in both proliferative and secretory endometrium were found in the current study. Although, decreased E-cadherin expression in endometrioid carcinoma compared with proliferative or secretory endometrium was similarly found in the current study. The mechanism of reduced E-cadherin positivity has yet to be fully understood; however, Saito *et al*. highlighted that the loss of E-cadherin positivity was caused by the promoter methylation of the E-cadherin gene.[22] In the current study, a significant difference in E-cadherin immunopositivity was found between endometrial atypical hyperplasia and proliferative or secretory endometrium, as well as between endometrioid carcinoma and proliferative or secretory endometrium. E-cadherin immunopositivity being significantly different between endometrial atypical hyperplasia and endometrioid carcinoma was also demonstrated. So far, no previous study has mentioned this difference in E-cadherin positivity between endometrial atypical hyperplasia and carcinoma.

β-catenin, which is encoded by the *CTNNB1* gene, is a subunit of the cadherin protein complex. It participates in the formation of adherens junctions that play a pivotal role in maintaining epithelial cell layers by regulating cellular adhesion and growth signals [Figure 1].[23]

Several studies have indicated that β-catenin has been implicated in the pathogenesis and progression of numerous human malignancies involving the Wnt pathway. As a signal transducer in the Wnt pathway, it induces targeted gene expression and cytoplasmic β-catenin accumulation.[24] Previous studies have demonstrated greater positivity of cytoplasmic β-catenin in the glandular cells of proliferative endometrium as compared with the secretory phase. These studies have also highlighted the nuclear positivity of β-catenin in the glandular cells of the proliferative and early secretory phase endometrium.[8,24] However, no difference in β-catenin immunopositivity was found between proliferative and secretory endometrium. Furthermore, no nuclear β-catenin immunopositivity was found in proliferative/secretory endometrium or endometrial atypical hyperplasia. Shih *et al*. revealed that nuclear β-catenin-positive cells lacked E-cadherin positivity, which indicated an inverse correlation between E-cadherin and nuclear β-catenin positivity.[8,25] This result was concordant with the current study, where 14% of endometrioid carcinoma cases exhibited nuclear β-catenin immunopositivity and a majority of them showed near total loss of membranous E-cadherin immunopositivity. The exact mechanisms behind this reduced positivity of E-cadherin at nuclear β-catenin positive sites are yet to be elucidated; however, one of them may be the nuclear translocation of β-catenin, which impairs the β-catenin/E-cadherin adherent junction complex, leading to E-cadherin release from the cell membrane.

The mechanisms of the nuclear accumulation of β-catenin have been reported to be responsible for the mutation of β-catenin and related genes. Studies on the Wnt pathway in colorectal cancers have demonstrated that β-catenin stabilisation and its significant accumulation in the cell, which were primarily attributed to the mutation of the *APC* (*adenomatosis polyposis coli*) or the β-catenin gene in the signalling pathway, result in the cell cycle progression in colorectal cancer.[26] The current study indicated decreasing membranous immunopositivity of β-catenin from proliferative/secretory endometrium to endometrial atypical hyperplasia and endometrioid carcinoma. Furthermore, the current study also highlighted significant differences in β-catenin immunopositivity between endometrial atypical hyperplasia and proliferative/secretory endometrium; endometrioid carcinoma and proliferative/secretory endometrium; and endometrial atypical hyperplasia and endometrioid carcinoma. In the current study, nuclear β-catenin positive cases of endometrioid carcinoma exhibited increased cytoplasmic c-Myc immunopositivity. Hence, both c-Myc and β-catenin were found to be upregulated in these cases of endometrioid carcinomas.

c-Myc is a nuclear DNA binding protein that is implicated in cell cycle regulation. The c-Myc protein amplifications in many human cancers were found to be associated with tumour aggressiveness and poor prognosis.[27] A cyclic variation in c-Myc positivity was reported by Odom *et al*., with higher expression in the proliferative phase than in the secretory phase.[28] In contrast to this finding, increased c-Myc immunopositivity in secretory endometrium compared with proliferative endometrium was observed in the current study. Bircan *et al.* demonstrated that the anti-c-Myc monoclonal antibody was detected both in the nucleus and the cytoplasm, which was concordant with the current study. Actively dividing the cells of the endometrium in the proliferative phase displayed a nuclear distribution, while the immunostaining was primarily cytoplasmic in the differentiated cells of the secretory phase.[29] They demonstrated cytoplasmic and perinuclear c-Myc positivity in 15.3% of endometrial cancers. Another study by Geisler *et al*. demonstrated both cytoplasmic and nuclear c-Myc immunopositivity in 75.2% and 66.9% of the cases of endometrial cancers, respectively.[30] In contrast, only cytoplasmic c-Myc immunopositivity was found in all cases of endometrioid carcinomas in the current study, along with few cases of proliferative and secretory endometrium, showing nuclear c-Myc immunopositivity. Increasing cytoplasmic immunopositivity of c-Myc from proliferative/secretory endometrium to endometrial atypical hyperplasia and endometrioid carcinoma was also found. Significant differences in immunopositivity were also observed between endometrial atypical hyperplasia and proliferative endometrium; endometrioid carcinoma and proliferative/secretory endometrium; and endometrial atypical hyperplasia and carcinoma. However, no significant difference in c-Myc immunopositivity between endometrial atypical hyperplasia and secretory endometrium was found.

Conclusion

Decreasing DKK1 immunopositivity from proliferative/secretory endometrium to endometrial atypical hyperplasia and endometrioid carcinoma indicates that *DKK1* is down regulated in endometrioid endometrial carcinoma. Immunoprofiles of DKK1 and the other markers associated with the Wnt signalling pathway explain the antagonistic role DKK1 plays in the Wnt signalling pathway in endometrial cancer. Therefore, DKK1 shows promise as a biomarker for screening the progression of endometrioid carcinoma. On the other hand, reactivation of the *DKK1* gene could be a valuable strategy for antagonizing the Wnt signalling pathway.

AUTHORS' CONTRIBUTION

AD and SM conceptualised and designed the study. SM drafted the manuscript. SK and NB performed a critical review and contributed with suggestions. SM and AD were involved in data collection, data entry, literature search and data analysis. All the authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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References

1. American Cancer Society. Cancer facts and figures 2021. From: https://doi.org/10.1057/978-1-349-96042-2_16551 Accessed: Oct 2022.
2. Lewis DR, Chen HS, Cockburn MG, Wu XC, Stroup AM, Midthune DN, et al. Early estimates of SEER cancer incidence, 2014. Cancer 2017; 123:2524–34. https://doi.org/10.1002/cncr.30630.
3. Kushner DM, Lurain JR, Fu TS, Fishman DA. Endometrial adenocarcinoma metastatic to the scalp: Case report and literature review. Gynecol Oncol 1997; 65:530–3. https://doi.org/10.1006/gyno.1997.4718.
4. Nath A, Mathur, P. Strengthening cancer surveillance in India: Role of the National Cancer Registry Programme. Indian J Surg Oncol 2022; 13:2–7. https://doi.org/10.1007/s13193-021-01473-8.
5. Colombo N, Preti E, Landoni F, Carinelli S, Colombo A, Marini C, et al. Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2011; 22:vi35–9. https://doi.org/10.1093/annonc/mdr374.
6. Vergote I, Amant F, Timmerman D. Should we screen for endometrial cancer? Lancet Oncol 2011; 12:4–5. https://doi.org/10.1016/s1470-2045(10)70280-1.
7. Bienz M, Clevers H. Linking colorectal cancer to Wnt signaling. Cell 2000; 103:311–20. https://doi.org/10.1016/s0092-8674(00)00122-7.
8. Shih HC, Shiozawa T, Miyamoto T, Kashima H, Feng YZ, Kurai M, et al. Immunohistochemical expression of E-cadherin and beta-catenin in the normal and malignant human endometrium: An inverse correlation between E-cadherin and nuclear beta-catenin expression. Anticancer Res 2004; 24:3843–50.
9. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004; 20:781–810. https://doi.org/10.1146/annurev.cellbio.20.010403.113126.
10. Mao J, Wang J, Liu B, Pan W, Farr GH 3rd, Flynn C, et al. Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. Mol Cell 2001; 7:801–9. https://doi.org/10.1016/s1097-2765(01)00224-6.
11. Peng S, Miao C, Li J, Fan X, Cao Y, Duan E. Dickkopf-1 induced apoptosis in human placental choriocarcinoma is independent of canonical Wnt signaling. Biochem Biophys Res Commun 2006; 350:641–7. https://doi.org/10.1016/j.bbrc.2006.09.087.

12. Yi N, Liao QP, Li T, Xiong Y.Novel expression profiles and invasiveness-related biology function of DKK1 in endometrial carcinoma. Oncol Rep 2009; 21:1421–7. https://doi.org/10.3892/or_00000370.

13. Pyo SW, Hashimoto M, Kim YS, Kim CH, Lee SH, Johnson KR, et al. Expression of E-cadherin, P-cadherin and N-cadherin in oral squamous cell carcinoma: Correlation with the clinicopathologic features and patient outcome. J Craniomaxillofac Surg 2007; 35:1–9. https://doi.org/10.1016/j.jcms.2006.11.004.

14. Kumar V, Abbas AK, Aster JC.Robbins and Cotran Pathologic Basis of Disease, 9th Ed. Philadelphia, USA: Elsevier Saunder, 2015. p.1014.

15. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15:10–17. https://doi.org/10.1016/0090-8258(83)90111-7.

16. Bansal N, Yendluri V, Wenham RM. The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. Cancer Control 2009; 16:8–13. https://doi.org/10.1177/107327480901600102.

17. Bafico A, Liu G, Yaniv A, Gazit A, Aaronson SA. Novel mechanism of Wnt signalling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. Nat Cell Biol 2001; 3:683–6. https://doi.org/10.1038/35083081.

18. Mao B, Wu W, Li Y, Hoppe D, Stannek P, Glinka A, et al. LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature 2001; 411:321–5. https://doi.org/10.1038/35077108.

19. Maniatis T. A ubiquitin ligase complex essential for the NF-kappaB, Wnt/Wingless, and Hedgehog signaling pathways. Genes Dev 1999; 13:505–10. https://doi.org/10.1101/gad.13.5.505.

20. Licchesi JD, Westra WH, Hooker CM, Machida EO, Baylin SB, Herman JG. Epigenetic alteration of Wnt pathway antagonists in progressive glandular neoplasia of the lung. Carcinogenesis 2008; 29:895–904. https://doi.org/10.1093/carcin/bgn017.

21. Fadare O, Reddy H, Wang J, Hileeto D, Schwartz PE, Zheng W. E-Cadherin and beta-Catenin expression in early stage cervical carcinoma: A tissue microarray study of 147 cases. World J Surg Oncol 2005; 3:38. https://doi.org/10.1186/1477-7819-3-38.

22. Saito T, Nishimura M, Yamasaki H, Kudo R. Hypermethylation in promoter region of E-cadherin gene is associated with tumor dedifferention and myometrial invasion in endometrial carcinoma. Cancer 2003;97:1002–9. https://doi.org/10.1002/cncr.11157.

23. Thompson MD, Monga SP. WNT/beta-catenin signaling in liver health and disease. Hepatology 2007; 45:1298–305. https://doi.org/10.1002/hep.21651.

24. Gumbiner BM. Signal transduction of beta-catenin. Curr Opin Cell Biol 1995; 7:634–40. https://doi.org/10.1016/0955-0674(95)80104-9.

25. Nei H, Saito T, Yamasaki H, Mizumoto H, Ito E, Kudo R. Nuclear localization of beta-catenin in normal and carcinogenic endometrium. Mol Carcinog 1999; 25:207–18. https://doi.org/10.1002/(sici)1098-2744(199907)25:3<207::aid-mc7>3.0.co;2-4.

26. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997; 275:1787–90. https://doi.org/10.1126/science.275.5307.1787.

27. Watson JV, Curling OM, Munn CF, Hudson CN. Oncogene expression in ovarian cancer: A pilot study of c-myc oncoprotein in serous papillary ovarian cancer. Gynecol Oncol 1987; 28:137–50. https://doi.org/10.1016/0090-8258(87)90207-1.

28. Odom LD, Barrett JM, Pantazis CG, Stoddard LD, McDonough PG. Immunocytochemical study of ras and myc proto-oncogene polypeptide expression in the human menstrual cycle. Am J Obstet Gynecol 1989; 161:1663–8. https://doi.org/10.1016/0002-9378(89)90946-0.

29. Bircan S, Ensari A, Ozturk S, Erdogan N, Dundar I, Ortac F. Immunohistochemical analysis of c-myc, c-jun and estrogen receptor in normal, hyperplastic and neoplastic endometrium. Pathol Oncol Res 2005; 11:32–9. https://doi.org/10.1007/bf03032403.

30. Geisler JP, Geisler HE, Manahan KJ, Miller GA, Wiemann MC, Zhou Z, et al. Nuclear and cytoplasmic c-myc staining in endometrial carcinoma and their relationship to survival. Int J Gynecol Cancer 2004; 14:133–7. https://doi.org/10.1136/ijgc-00009577-200401000-00018.