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Original Research Article

Immunohistochemical expression of SNAIL and SLUG in endometrioid endometrial cancer and precursor lesions

Sunita Yadav[1]*, Annu Makker[2], Seema Nayak[3], Preeti Agarwal[4], Uma Singh[5], Madhu Mati Goel[3]

[1]Department of Pathology, Hind Institute of Medical Sciences, Barabanki, Uttar Pradesh, India
[2]Department of Biochemistry, Saraswati Medical College, Unnao, Uttar Pradesh, India
[3]Department of Laboratory Medicine, Medanta Hospital Lucknow, Uttar Pradesh, India
[4]Department of Pathology, KGMU, Lucknow, Uttar Pradesh, India
[5]Department of Maternal Health, TSU, Uttar Pradesh, India

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***Correspondence:**
Dr. Sunita Yadav,
E-mail: sunitayadavpari@gmail.com

ABSTRACT

Background: Endometrioid endometrial carcinoma is one of the most prevalent gynecologic cancers. The epithelial-mesenchymal transition is a multistep process involved in the development of cells that results in disappearance of intercellular adhesion in the epithelium and acquiring mesenchymal properties, hence influence cancer progression and metastasis. Loss of intercellular adhesion can be activated by different mechanisms, including transcriptional repression. However, the expression of transcriptional repressors in EEC and precursor lesions remain to be investigated. In the present study, we evaluated the immunohistochemical expression of proteins of transcriptional after repression snail and slug in EEC and its precursor lesions.
Methods: It was a prospective nested case-control study on women from 35 to 70 years of age. Endometrial biopsies were obtained and processed for routine histological diagnosis. Immunohistochemical expression of snail and slug were evaluated.
Results: A total of 39 EEC cases, 37 Endometrial hyperplasia and 19 normal controls were included in this study. Expression of snail was positive in 77% (30/39) and slug in 82% (32/39) of EEC cases while in precancer group snail was positive in 76% (28/37) and slug in 68% (25/37). In normal control snail was positive in 32% (6/19) and SLUG in 21% (4/19).
Conclusion: Up-regulation of snail & slug observed in both precancer and cancer cases, suggesting their involvement from an early stage of carcinogenesis. Therefore therapies targeted at transcriptional repressors at an early stage of carcinogenesis, i.e., at precancerous lesions, could play a valuable role in reducing cancer progression and metastasis.

Key words: Immunohistochemistry, Endometroid endometrial carcinoma, Endometrial hyperplasia, Slug, Snail

INTRODUCTION

Endometrial carcinomas (EC) are one of the most prevalent gynecologic cancers.[1] It is most common invasive cancer of the uterine corpus, globally. About 7% of all invasive cancer in women are contributed by endometrial carcinoma.[2] In comparison to other parts of the world, Asian women have a lower incidence of EC. But recent research has showed that it is increasing in these countries, including India.[3,4] Clinicopathologically, EC is divided into two categories: type I and type II.

Endometrioid endometrial carcinoma (EEC) i.e., type I constitutes 80% to 85% of all EC, are estrogen dependent and arises from a background of endometrial hyperplasia (EH).[5,6] Most of the type I EC are well differentiated.[7] Type I EC often associated with diabetes mellitus, obesity, hypertension, nulliparity, infertility and unopposed estrogen stimulation.[7] Type II carcinomas are estrogen independent, usually arise in the setting of endometrial atrophy and are poorly differentiated.[8] It accounts for approximately 15% of all EC cases.[8] EMT is a multistep process involved in the development of cells that results in disappearance of intercellular adhesion in the epithelium, acquiring mesenchymal properties and the migratory phenotype, and increased resistance to apoptosis. EMT has also been shown to influence cancer progression and metastasis.[9] Invasion and metastasis are the major cause of cancer-related morbidity and mortality hence are the subject of intense investigation. The adhesion molecules play a critical role in the maintenance of epithelial cell to cell adhesion and normal histologic structure.[10] The first step in initiation of the EMT, which also stimulates mesenchymal genes, is loss of cellular adhesion due to the decreased expression of E-cadherin, evolving from the activities of several major transcription factor, including SNAIL, SLUG, TWIST and ZEB1.[11-13] However, the expression of proteins related to transcriptional repression in EEC and precursor lesions i.e., endometrial hyperplasia are under-investigated. In the present study, we immunohisto-chemically evaluated the expression levels of SNAIL and SLUG in EEC and its precursor lesions.

METHODS

Study type and design

It was a prospective nested case control study. The study was carried out at department of pathology, King George's Medical University, Lucknow, Uttar Pradesh, India. The study was conducted during February 2017 to March 2018. A total of 95 endometrial biopsies including surgically resected specimens and clinical parameters were included. Biopsies were classified as normal proliferative endometrium (N=19), hyperplastic endometrium (N=37) and endometrioid endometrial carcinoma (N=39) as per WHO criteria.[14] Prior to the enrolment in the study, a signed informed consent was obtained from all the study participants. Patients diagnosed with any other malignancy outside the uterus and all endometrial malignancy other than EEC were excluded.

Laboratory techniques and procedure

Histopathological diagnosis: The tissues were collected in 10% formalin and processed according to the paraffin embedding standard procedure in a tissue processor and 5–6-micron thick sections were stained with hematoxylin and eosin (H & E) stain for the histopathology reporting. Immunohistochemistry and scoring: Immunohisto-chemistry were performed on paraffin embedded sections from normal, hyperplasias and EEC lesions. Antigen retrieval was done in EZ antigen retriever system (BioGenex, USA) in citrate buffer (pH 6.0) for SNAIL and SLUG, at 98°C for 15 minutes. Sections were stained with purified rabbit polyclonal SNAIL antibody (Abgent AP2054a) at 1:10 dilution and purified rabbit polyclonal SLUG antibody (Abgent AP2053a) at 1:10 dilution and incubated 1 hour 30 minutes at 25°C. Then washed and incubated with Novolink Polymer (Leica Biosystems Newcastle Ltd) at room temperature for one hour, after that application of 3,3'-diaminobenzidine tetra hydrochloride solution (DAB) (Table 1). Semiquantitative scoring was done for assessing the intensity and proportion of cells stained for SNAIL and SLUG. Immunointensity was scored on a scale of 0-4; 0: no staining, 1: light staining, 2: low intermediate, 3: high intermediate and 4: darkest brown stain. On a scale of 1-4; the proportion of staining was scored as 1: 0-25% of cells stain positive, 2: 26-50% of cells stain positive, 3: 51-75% of cells stain positive and 4: 76-100% of cells stained positive. Immunointensity and immunopositivity scores were multiplied giving results that ranged from 0 to 16. Final estimation was done as negative for sections with a score of 0-3, and positive for sections with score ≥4.[15]

Statistical analysis

SPSS software version 24 was used for the analysis of results. To compare the categorical variables Pearson's Chi square tests were used. Statistical significance was defined as p value <0.05.

RESULTS

A total of 39 cases of EEC, 37 cases of EH comprising of 27 EH without atypia and 10 EH with atypia along with 19 normal proliferative endometrial cases were included in this study. Age of women ranged from 35 years to 70 years with mean±SD is 49.11±11.67. Most frequent complaints in EEC patients were bleeding per vaginum (95%) and in precancer cases, abnormal uterine bleeding (43%). In cancer group 62% and in precancer group 65% were overweight. History of menopause was present in 69% of EEC cases and in precancer cases 86% were premenopausal. Parity status of more than two parity was observed in 65% of precancer and 67% of cancer cases (Table 2).

Immunohistochemical expression

In this study, expression of SNAIL was positive in 77% (30/39) and SLUG in 82% (32/39) of EEC cases while in precancer group SNAIL was positive in 76% (28/37) and SLUG in 68% (25/37). In normal control SNAIL was positive in 32% (6/19) and SLUG in 21% (4/19). Within the precancer group, SNAIL and SLUG showed significant increase in expression in both subgroups (endometrial hyperplasia with atypia and without atypia) (Table 3). Microphotographs of representative immunostained section of various lesions are shown in plate (Figure 1). Statistically significant increase in immunohistochemical

expression of SNAIL and SLUG was observed in glandular epithelium of precancer (χ^2=10.23, p value=0.001), (χ2=10.87, p value=0.001) and cancer (χ^2=11.15, p value=0.001), (χ^2=20.19, p value<0.001) as compared to control.

Table 1: Details of antibodies, their dilutions, antigen retrieval buffer, temperature and time conditions, staining pattern.

Primary Antibody	Dilution	Antigen retrieval buffer and conditions	Incubation with primary antibody	Secondary detection kit	Cellular localization
SNAIL, (Abgent), (AP2054a)	1:10	Citrate buffer (pH 6.0) 15 min at 98^{0}C	1h 30 min at 25^{o}C	Novolink Polymer (Leica Biosystems Newcastle Ltd	Cytoplasmic
SLUG, (Abgent), (AP2053a)	1:10	Citrate buffer (pH 6.0) 15 min at 98^{0}C	1h 30 min at 25^{o}C	Novolink Polymer (Leica Biosystems Newcastle Ltd	Nuclear and cytoplasmic

Table 2: Clinical and demographic details of study population.

Variables	Control (N=19)	Precancer (N=37)	Cancer (N=39)
Age (year)			
Mean±SD	39.63±8.03	45.51±8.61	57.13±10.62
Median	42	46	60
Chief complaints (%)			
Bleeding PV	26	43	95
Pain in abdomen	26	30	32
UV prolapse	-	-	11
Vaginal discharge	-	23	11
Vaginal Pain	-	4	5
Menopausal status, N (%)			
No	19 (100)	32 (86)	12 (31)
Yes	0 (0)	5 (14)	27 (69)
Body mass index, N (%)			
Normal (18.5-24.9)	18 (95)	11 (30)	13 (33)
Obese (>30)	0 (0)	4 (11)	5 (13)
Pre obesity (>25-29.9)	1 (5)	20 (54)	19 (49)
Underweight <18)	0 (0)	2 (5)	2 (5)
Parity, N (%)			
1≤	3 (16)	13 (35)	13 (33)
2≥	16 (84)	24 (65)	26 (67)

Table 3: Immunohistochemical analysis of SNAIL and SLUG.

IHC Markers			SNAIL		SLUG	
Expression			**Positive**	**Negative**	**Positive**	**Negative**
Control (N=19), frequency; %			6; 32	13; 68	4; 21	15; 79
Precancer (N=37)	EH without atypia (N=27)	Frequency; %	20; 74	7; 26	15; 56	12; 44
		X2; p value	8.195; 0.0209		0.0326	
	EH with atypia (N=10)	Frequency; %	8; 80	2; 20	10; 100	0; 0
		P value	0.0209		0.0000	
	Total precancer (N=37)	Frequency; %	28; 76	9; 24	25; 68	12; 32
		X2; p value	10.23; 0.001		10.87; 0.001	
Cancer (N=39)		Frequency; %	30; 77	9; 23	32; 82	7; 18
		X2; p value	11.15; 0.001		20.19; <0.001	

On comparison with non-myoinvasive tumors, those with deep myometrial invasion had increased expression of SNAIL and SLUG but this observation was statistically insignificant.

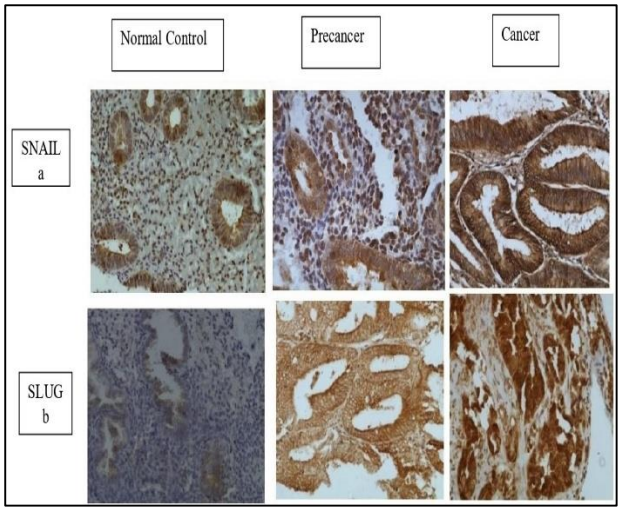

Figure 1: Immunohistochemical localization of; a) SNAIL in normal proliferative endometrium showing weak to moderate cytoplasmic staining, in precancerous endometrium (Endometrial Hyperplasia) showing moderate to strong cytoplasmic staining and in endometrioid endometrial carcinoma showing strong cytoplasmic staining in glandular epithelium, b) SLUG in normal proliferative endometrium showing weak cytoplasmic and nuclear staining, in precancerous endometrium (Endometrial Hyperplasia) showing moderate nuclear and cytoplasmic staining and in endometrioid endometrial carcinoma showing strong nuclear and cytoplasmic staining in glandular epithelium.

DISCUSSION

We analyzed immunohistochemical expression of major transcription factors SNAIL & SLUG in normal, precancerous and cancerous endometrial lesions. In the present study 77% of EEC, 76% of precancer groups and 32% (6/19) normal control showed cytoplasmic expression of SNAIL in glandular epithelium. Increase in immunohistochemical expression of SNAIL was statistically significant in precancer and cancer as compared to controls. Sadlecki et al found 84.5% of EC specimens with positive expression of SNAIL.[16] Hence, upregulation of SNAIL also plays an important role in tumor growth and progression to metastasis. Supernat et al however, found decreased SNAIL expression in EC samples, this may be because of their small sample size and they included only one control in their study.[17] The nuclear expression of SLUG was observed in 82% (32/39) of EEC, 68% (25/37) of precancer and 21% (4/19) of normal control cases in our study. Statistically significant increase in expression of SLUG were found in precancer (p value=0.001) and cancer (p value<0.001) cases as compared to normal control. Zhu et al studied immuohistochemical expression of SLUG in 124 EC and 35 normal endometrial tissue.[18] They found significant increase (61.3%, 76/124) in SLUG expression in EC as compared to normal controls. Sadlecki et al found 92.2% of EC with positive expression of SLUG.[16] Upregulation of SLUG has important role in proliferation, invasion and migration, hence acts as a promoter of EMT. They also observed that SLUG and SNAIL expression were significantly higher in type II EC when it was compared to type I EC. Transcriptional activators for EMT i.e. SNAIL and SLUG expression were increased in endometrial precancer and cancer as compared to normal control, suggesting involvement of EMT since early stages of cancer. SNAIL and SLUG can down-regulate E-cadherin and thus regulate EMT.[19] However there is no therapy available right now that targets SNAIL and SLUG. A drug that targets Netrin-1 inhibits the EMT in cancer, that is stimulated by the molecule Netrin-1, which is expressed by tumor cells in different kinds of cancer.[20] Upregulation of SNAIL and SLUG in precancer and cancer cases as compared to normal control may regulate and control the EMT hence play an important role in tumor occurrence, development, invasion and metastasis. Our results suggest that upregulation of SNAIL and SLUG in EEC and EH may be an important marker of EMT and act as inducers for tumorigenesis. The present study is not free from limitations. Since this was a time bound study, sample size was small. It would have been better if authors could be able to add grading of EC and follow up data.

CONCLUSION

Up-regulation of SNAIL & SLUG observed in both precancer and cancer cases as compared to normal control, suggesting their involvement from an early stage of carcinogenesis. Therefore therapies targeted at transcriptional proteins SNAIL and SLUG at an early stage of carcinogenesis, i.e., at precancerous lesions, could play a valuable role in reducing cancer progression and metastasis.

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