

## TUBAL SCOUTs, STILs, STICs and p53 Signatures: Understanding the new language in ovarian serous carcinoma

Aneeta Jassar<sup>1,\*</sup>, Deepak Patil<sup>2</sup>

<sup>1</sup>Senior Advisor & HOD, Dept. of Pathology, <sup>2</sup>Specialist, Dept. of Gynecology & Obstetrics, Military Hospital, Jalandhar, Punjab

**\*Corresponding Author:**

Email: draneetajassar@gmail.com, aneetarun@yahoo.in

### Abstract

**Background:** Conventionally, carcinomas of the ovary have been thought to arise de novo from the ovarian surface. However recent studies have indicated that putative 'precursor' changes are present in the fimbrial ends of fallopian tubes.

**Aims:** The study was aimed to identify histological changes in the fimbrial ends of fallopian tubes across three sets of patients.

**Settings and design:** Histological sections from fimbrial ends of fallopian tubes from a total of 14 women who underwent prophylactic salpingo-oophorectomy for non-neoplastic diseases, twelve who were treated for non-serous ovarian carcinoma and nine consecutive cases of ovarian serous carcinoma cases over a three year period from a tertiary care hospital were studied.

**Methods and Materials:** Sections were stained with hematoxylin-eosin and p53 immunohistochemical staining.

**Statistical analysis used:** One-way ANOVA (Analysis of Variance).

**Results:** Histological changes in the fimbriae ranging from SCOUT to STIL and STIC and p53 signatures were found across all three populations but were multifocal and significantly higher in cases of ovarian serous carcinoma.

**Conclusions:** Our study identified statistically significant differences in histological changes and p53 immunostaining in the fimbrial end of fallopian tubes across three populations of patients.

**Keywords:** Ovarian serous carcinoma, Fallopian tube, Secretory Cell Outgrowths (SCOUTs), Serous Tubal Intermediate Lesions (STILs), Serous Tubal Intraepithelial Carcinoma (STIC), p53 signatures

### Introduction

Ovarian cancer afflicts nearly 2,00,000 women globally every year. Despite its relative low incidence, it is the seventh leading cause of cancer-related deaths among women due to its high death-to-incidence ratio.<sup>(1)</sup> Among all ovarian cancers, the outcome of ovarian serous carcinoma (OSC) is the most dismal, largely because efforts at early detection have not been very successful in the past. Conventionally, serous carcinomas of the ovary have been thought to arise de novo from ovarian surface epithelium and/or epithelial inclusion glands. However the traditional 'incessant ovulation'<sup>(2)</sup> and 'metaplastic' theories have left many unanswered questions. A convincing precursor lesion and a plausible carcinogenesis model (with associated molecular events) have evaded workers up to now. Research over the last few years has, unexpectedly, thrown up a plethora of histologically identifiable 'precursor' lesions not of ovarian but extraovarian origin.<sup>(3)</sup> These are in the fallopian tube and include Secretory Cell Outgrowths (SCOUTs), Serous Tubal Intermediate Lesions (STILs) and Serous Tubal Intraepithelial Carcinoma (STIC). Immunohistochemistry for the apoptosis protein p53 has added further information about events at the molecular level and thrown up new terms like 'p53 Signatures'.

This study sought to study the spectrum of putative precursor tubal lesions and p53 alterations in fallopian tubes of normal patients and in those with serous and non-serous ovarian carcinoma, with an aim to

understand the pathogenesis pathway in ovarian serous carcinoma.

### Materials and Methods

The study was approved by the institutional review board. The first goal was to evaluate histological changes in the fimbrial and proximal ends of fallopian tubes in three different populations. The second was to determine the prevalence of p53 signatures in these populations and if these overlapped with the histological changes.

Subjects consisted of consecutively treated women in a tertiary care hospital over a three-year period. They were divided into three groups, including (1) Fourteen women who underwent prophylactic salpingo-oophorectomy for non-neoplastic diseases (like endometriosis, adenomyosis, uterine leiomyomata etc), (2) Twelve consecutive women treated for non-serous ovarian carcinoma (NSC; mucinous or endometrioid) and (3) Nine consecutive cases of ovarian serous carcinoma (OSC).

Fallopian tubes from all three populations were subjected to serial longitudinal sectioning at proximal (1inch from the fimbrial end) and at the fimbrial end. All sections were stained with hematoxylin and eosin and examined histologically.

The lining epithelium of a normal fallopian tube shows alternating pseudo-stratified ciliated and secretory cells. The secretory cell is considered to be the precursor of the former, undergoing ciliation under hormonal control. SCOUT was diagnosed if there were discrete linear segments with a continuous population

of 30 or more secretory cells without an intervening ciliated cell.

Presence of three or more of the following histological features were taken as criteria for diagnosis of Serous Tubal Intra-epithelial Carcinoma (STIC):

- Irregular luminal surface,
- Epithelial stratification (including loss of cellular polarity and/or presence of nuclear moulding),
- Cellular and/or nuclear pleomorphism,
- Nuclear enlargement (high N:C ratio),
- Irregular nuclear chromatin (hyperchromasia/vesicular),
- Prominent nucleoli,
- Mitotic figures and/or apoptotic bodies.

Lesions were labelled as Serous Tubal Intermediate Lesions (STILs) if they had less than three of the above features.

A monoclonal antibody to p53, targeting an epitope in aminoacids 21-25 of the protein, was used to localize p53 protein (Oncogene Research Products, San Diego, CA, USA). Adequate controls were used. 'p53 signatures' were defined as small segments of strongly p53-positive, benign-appearing epithelium.

- *Positive p53*
  - Strong nuclear staining for at least 12 consecutive nuclei or > 75% of cells (this excluded physiological staining that is usually limited to no more than 2 or 3 consecutive nuclei in the tubal epithelium), or
  - Complete absence of staining (Null p53)
- *Negative p53*
  - Weakly staining cells or focal/patchy expression (Wild p53).

**Statistical Analysis:** The null hypothesis was tested using the One-way or One-factor ANOVA (Analysis of Variance) test across three sets of histological changes, viz. SCOUT, STIL and STIC as well as for p53 staining across the three patient groups. P value was calculated using version 4.0 of the Free Statistics Calculators (<http://www.danielsoper.com/statcalc/default.aspx>).

## Results

All H & E sections were evaluated for SCOUT, STIL and STIC as per criteria described above. Attributes of p53 signatures were analyzed and where appropriate, compared with normal epithelium and STIC with a view to analyze:

- frequency in the three patient groups;
- localization (fimbrial vs proximal tube);
- focality (focal/multifocal).

SCOUTs were found in 29% of normal tubes. However, in the absence of testing for BRCA gene mutations, it is difficult to assess if these women represented a truly increased risk for ovarian carcinoma. SCOUT and loss of ciliated cells at fimbrial ends was found in 42% (5/12) cases of NSC and in 89% (8/9) cases of OSC. Atypical intermediate lesions (STILs)

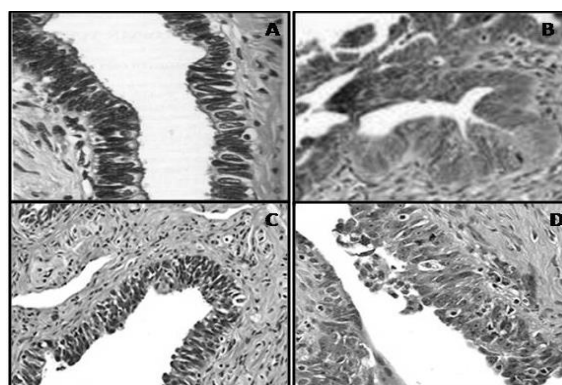
were not found in normal tubes but present in 25% (3/12) cases of NSC and in 7 cases of OSC (78%), where they were also multifocal (Table 1). Changes consistent with STIC were seen in 42% of NSC and 89% of OSC (where they were also multifocal) and its preferred site was the fimbriated end in close proximity to the ovarian surface (Table 1 and Fig. 1). P value using the one-way ANOVA test was 0.00 (or less than 0.05) and thus the histological changes of SCOUT, STIC and STIL and p53 staining were significantly more in cases of ovarian serous carcinoma.

p53 signatures were found across all three populations, though they were focal and found only in fimbrial ends in 21% of normal women and in 33% of those with NSC, as compared to all (9/9) cases of OSC. In the eight cases that showed STIC, p53 signatures were found predominantly in foci of STIC (Table 2 and Fig. 2).

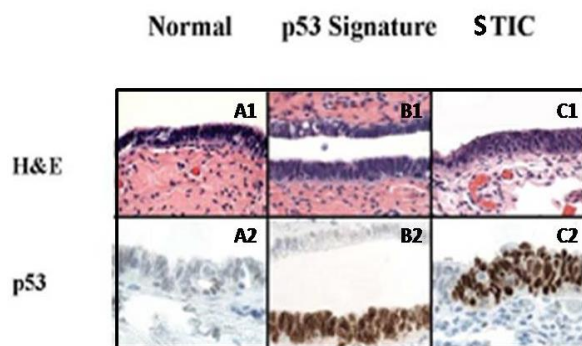
**Table 1: Frequency of precursor lesions in three populations of patients**

	Normal (n=14)	Non-Serous Ovarian Cancer (n=12)	Ovarian Serous Cancer (n=9)
SCOUT	4 (29%)	5 (42%)	8 (89%)
STIL	0	3 (25%)	7 (78%) †
STIC	0	5 (42%)	8 (89%) †

† Multifocal



**Fig. 1 H&E appearance of distal ends of fallopian tubes (A) Normal fallopian tube with ciliated and non-ciliated (secretory) cells. (B) Homogeneous secretory cell outgrowth (SCOUT) with absence of ciliated differentiation. (C) Serous Tubal Intermediate Lesion (STIL) with epithelial stratification and mild atypia. (D) Serous Tubal Intra-epithelial Carcinoma (STIC) showing marked atypia and mitotic figures.**



**Fig. 2: Comparison of H&E and p53 staining A1 & A2: Wild-type p53 staining in histologically normal fallopian tube. B1 & B2: p53 signature (diffuse staining) in a focus of SCOUT. C1 & C2: Diffuse and strong p53 staining in STIC.**

**Table 2: Localization and p53 signatures in three patient populations**

Subjects	Morphological changes in Fallopian tubes		Frequency of p53 signatures	
	Proximal ends	Fimbriated ends	Proximal ends	Fimbriated ends
Normal (n=14)	0	9 (64%)	0	3 (21%)
NSC (n=12)	1 (8%)	10 (83%)	0	4 (33%)
OSC (n=9)	1 (11%)	8 (89%)	2 (22%)	9 (100%)

## Discussion

The study was prompted by the recent discovery of the fallopian tube as the site for precursor changes in serous ovarian carcinoma. The protocol of systematic sectioning of the tubes from three patient populations was followed and sections stained with H & E and p53 to look for precursor lesions and pattern of p53 staining.<sup>(4)</sup>

This study revealed STIC in 42% of NSCs and in 89% cases of OSC. Kindelberg et al had studied a range of tumors in women, almost all of which had no history of germline BRCA mutations and identified STIC in 20 of 43 (47%) tumors classified as ovarian in origin.<sup>(5)</sup> p53 signatures were found in the fimbriae in all cases (100%) and in the proximal ends in 22% of the cases. Both STIC and p53 signatures were localised predominantly to the fimbrial ends and commonly associated together (in 8/9 cases). The ratio of fimbrial to proximal location for p53 signature in the study by Lee et al and the current study was 4.2:1 and 4.5:1 respectively.<sup>(6)</sup> p53 signatures are also found, albeit less commonly, in non-neoplastic tubes, suggesting ovulation-related oxidative stress, which may be the earliest molecular change in the cancer pathway.<sup>(7)</sup>

In this study, SCOUT was found 29% of normal tubes and in 42% of NSC and in almost all (89%) patients with OSC. SCOUT has been recognized as the earliest histological change.<sup>(8)</sup> In the model elucidated

by Mehra et al, tubal epithelium at the fimbrial end experiences oxidative damage (either from ovulation or inflammation), shows overgrowth of secretory cells and loss of ciliated cells with over-accumulation of p53 protein over time.<sup>(8)</sup> Some workers have also demonstrated p53(-) SCOUTs and whether these are also part of the sequence in carcinogenesis merits further investigation.<sup>(9)</sup> PAX-2, a marker expressed in secretory cells of normal tubes, has shown reduced expression in both p53(-) SCOUTs and p53 signatures.<sup>(10)</sup>

Criteria for atypical intermediate lesions of the tube (STILs) have not yet been elucidated clearly. These most likely represent intermediate steps in the pathogenesis and are best reported as a descriptive diagnosis, with a comment that the lesion is insufficient for a diagnosis of STIC.<sup>(8)</sup> As the morphologic spectrum of precursor lesions is wide and standardized criteria are still in their infancy, studies might differ slightly in distinguishing normal mucosa from SCOUT, STIL and STIC.<sup>(11)</sup> As a consequence, subjective variability in diagnosis exists between pathologists. Visvanathan et al utilized a point-score system to achieve reproducibility on histology but found that inter-observer agreement was only fair ( $k=0.39$ ) but incorporation of IHC improved reproducibility.<sup>(12)</sup> Some workers have found that combining the diagnoses into two categories (STIC versus non-STIC) improved the diagnostic reproducibility for tubal mucosal lesions.<sup>(13)</sup> Kuhn et al found that strong diffuse staining correlated with a missense TP53 mutation, complete absence of staining meant null mutations while weak and patchy staining generally corresponded to wild type TP53.<sup>(14)</sup> Vang et al have proposed a diagnostic algorithm which combines histological features with IHC for p53 and Ki-67 proliferation index, in order to substantiate the unequivocal diagnosis of STIC with an abnormal p53 expression ('All or none') and high Ki-67 index.<sup>(11)</sup> Ki-67 can be a useful adjunct to p53, especially as it has shown substantial interobserver reproducibility in two studies.<sup>(11,15)</sup> Kuhn et al used a cut-off of >10% positive cells to report low or high Ki-67 proliferation and showed that normal fallopian tubes and STICs have mean Ki-67 indices of <1-3% and 36-72% respectively.<sup>(15)</sup>

Lee et al have experimented with novel markers like  $\gamma$ -H2AX (a phosphorylated form of the histone H2AX) and showed that all precursors as well as OSC are strongly positive.<sup>(6)</sup> To identify complete loss of p53 expression ('null p53'), some investigators have applied genome-wide analysis and identified laminin  $\gamma$ 1 as one of the proteins expressed ubiquitously in STICs and serous carcinoma even in null p53.<sup>(16)</sup> This suggests that, in future, laminin  $\gamma$ 1 may emerge as a useful biomarker, bridging the gap between STICs on one hand and loss of p53 expression and low Ki-67 proliferation index on the other.

The limitations of this study are the small sample size and the unknown BRCA status of patients.

### Conclusion

The emerging pathogenesis model of OSC has important clinical implications and future preventive measures may include removal of entire tube with all hysterectomies as well as fimbriectomy (and not simple tubal ligation) for sterilization.<sup>(17)</sup> Given this scenario, pathologists must be able to recognise and accurately diagnose putative tubal precursor lesions like SCOUTs, STILs and STICs. Immunostaining for p53 and Ki-67 can improve reproducibility and are recommended. Future studies should determine how novel markers ( $\gamma$  H2AX,  $\gamma$ 1 laminin, HMGA2) and molecular alterations might be included in the diagnostic algorithm for tubal precursor lesions.

**Sources of support:** Institutional support

**Acknowledgement:** Department of Obstetrics and Gynecology (Military Hospital Jalandhar) for general support.

**Disclosure/ Conflict of interest:** The authors have none.

### References

1. Permuth-Wey J, Sellers TA. Epidemiology of ovarian cancer. *Methods Mol. Biol.* 2009;472:413-37.
2. Fathalla MF. Incessant ovulation – a factor in ovarian neoplasia? *Lancet* 1971;2:163.
3. Kurman RJ, Shih I. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer – shifting the paradigm. *Hum. Pathol.* 2011;42:918-31.
4. Medeiros F, Muto MG, Lee Y, Elvin JA, Callahan MJ, Feltmate C, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am J Surg Pathol* 2006;30:230–6.
5. Kindelberger D, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, et al. Intraepithelial carcinoma of the fimbrial and pelvic serous carcinoma: evidence for a causal relationship. *Am J Surg Pathol* 2007;31(2):161-9.
6. Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, Garber J, Birch C, Mou H, Gordon RW, Cramer DW, McKeon FD, Crum CP. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol* 2007;211:26-35.
7. Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature* 2005;434:907–13.
8. Mehra K, Mehrad M, Chen E, Ning G, Drapkin R, McKeon FD et al. STICs, SCOUTs and p53 signatures: a new language for pelvic serous carcinogenesis. *Frontiers in Bioscience* 2011;1:625-34.
9. Leitao MM, Soslow RA, Baergen RN, Olvera N, Arroyo C, Boyd J. Mutation and expression of the TP53 gene in early stage epithelial ovarian carcinoma. *Gynecol Oncol* 2004;93:301–6.
10. Chen EY, Mehra KK, Mehrad M, Ning G, Miron A, Mutter GL et al. Secretory cell outgrowth, PAX2 and serous carcinogenesis in fallopian tube. *J Pathol* 2010;222(1):110-6.
11. Vang R, Shih I, Kurman RJ. Fallopian tube precursors of ovarian low- and high-grade serous neoplasms. *Histopathology* 2013;62:44-58.
12. Visvanathan K, Vang R, Shaw P et al. Diagnosis of serous tubal intraepithelial carcinoma based on morphologic and immunohistochemical features: a reproducibility study. *Am J Surg Pathol* 2011;35:1766-75.
13. Li H, Lu Z, Shen K, Cheng W, Malpica A, Zhang J et al. Advances in serous tubal intraepithelial carcinoma: correlation with high grade serous carcinoma and ovarian carcinogenesis. *Int J Clin Exp Pathol.* 2014;7(3):848–57.
14. Kuhn E, Kurman RJ, Vang R, Sehdev AS, Han G, Soslow R et al. TP53 mutations in serous tubal intraepithelial carcinoma and concurrent pelvic high-grade serous carcinoma: evidence supporting the clonal relationship of the two lesions. *J Pathol* 2012;226:421-6.
15. Kuhn E, Kurman RJ, Sehdev AS, Shih I. Ki-67 labelling index as an adjunct in the diagnosis of serous tubal intraepithelial carcinoma. *IntJ Gynecol Pathol* 2012;31:416-22.
16. Kuhn E, Kurman RJ, Soslow RA et al. The diagnostic and biological implications of laminin expression in serous tubal intraepithelial carcinoma. *Am J Surg Pathol* 2012;36(12):1826-34.
17. Dietl J, Wischhusen J, Hausler SF. The post-reproductive fallopian tube: better removed? *Hum Reprod* 2011;26:2918-24.