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STICS, SCOUTs and p53 signatures; a new language for pelvic serous carcinogenesis

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1. ABSTRACT

The events leading to the most common and most lethal ovarian carcinoma – high grade serous carcinoma – have been poorly understood. However, the detailed pathologic study of asymptomatic women with germ-line BRCA 1 or BRCA2 (BRCA+) mutations has unearthed an early malignancy, serous tubal intraepithelial carcinomas (STIC), which has linked many peritoneal and ovarian serous carcinomas to the fimbria. The distinction between high-grade serous and endometrioid carcinomas continues to narrow, with shared alterations in expression of pTEN, PAX2 and p53. Moreover, the discovery of clonal alterations in p53 in benign tubal epithelium, – p53 signatures – has established a foundation for a serous cancer precursor in the fimbria. We have expanded this entity to include a "generic" secretory cell outgrowth (SCOUT), in the fallopian tube that is associated with altered PAX2. As the repertoire of gene alterations is expanded and its link to serous carcinogenesis clarified, a cogent pathway to high-grade Müllerian carcinomas will emerge. This will challenge conventional thinking about ovarian carcinogenesis but will provide a new template for studies of ovarian cancer prevention.

2. INTRODUCTION

Cancer of the ovary afflicts nearly 204,000 women worldwide each year across the globe, including approximately 23,000 Americans. Despite its relative low incidence rate, ovarian cancer is an extremely lethal disease, killing 125,000 women. It is the seventh leading cause of cancer-related deaths among women. In the United States, ovarian cancer ranks as the fifth deadliest malignancy among women, with an estimated 15,500 deaths per year. Importantly, ovarian cancer had the highest death-to-incidence ratio, exceeding even that of lung cancer, due principally to the fact that most patients (75%) present with advanced stage (III/IV) tumors, for which the 5 year survival rate is 30%. The outcome for serous carcinomas is the worst, as this tumor presents with extra-ovarian involvement in over 80% of cases. Surgery and chemotherapy will produce a complete response in 70% but relapse rates are high and the asymptomatic interval following cheomotherapy can be dishearteningly brief. The number of cancers and cancer-related deaths in the past decade has increased 30 and 18%, respectively. (1-4).

2.1. Traditional models of ovarian carcinogenesis

Epithelial ovarian malignancies are presumed to arise through a well-defined progression of genetic and histologic changes mediating the transition from normal tissues through precursor spectra, culminating in malignancy with metastatic potential. This model is rooted in the study of gastrointestinal cancers where such a progression has been observed at the tissue and molecular levels. (5,6) Ovarian cancers have not fit as cleanly into this model, because they are not a uniform tumor group. The term 'ovarian' cancer refers not to a single disease, but to a diverse group of malignancies affecting the ovary and other sites including peritoneum and fallopian tube. They are, and can be subclassified as serous, endometrioid, mucinous and clear cell types. Moreover, the 'normal' mucosal analogues to these histologies have been a subject of debate, ranging from ovarian surface epithelium to ovarian cortical inclusions to salpingeal epithelium and even epithelial inclusions on the peritoneal surface. These potential sources of malignancy will be teased apart to some degree as we address the different sites of origin and their respective precursor spectra and the concept of latent precursor unfolds (7, 8)

The Müllerian duct is an invagination of coelomic epithelium which eventually forms the fallopian tubes, uterus and endocervix. There are several possible sources of Müllerian epithelium in the ovary. Either endometrial or tubal epithelium is relocated to the ovarian surface by exfoliation, direct contact or adhesions (the 'transfer model') or the surface cells are induced to transform via metaplasia (the 'metaplasia model'). (9-11) In the metaplasia model the ovarian surface epithelium (OSE) shares a common embryologic origin with epithelia of Müllerian duct-derived tissues; (12,13) and has a phenotypic plasticity that can be demonstrated by its reactivity (immunostaining) with antibodies to both epithelial (cytokeratin, laminin and collagen IV) and mesothelial (vimentin, collagen I and III) antigens. *In-vitro* (cell culture), OSE cells can be induced to shift between epithelial and mesenchymal morphology. (13) The resulting surface epithelia express estrogen, progesterone and androgen receptors. (14) Carcinoma could arise in metaplastic epithelium through additional, possibly hormonally driven, events. (15 16 17) Tubal or endometrial metaplasia or transfer from their respective organs could also account for similarities of ovarian carcinoma to other gynecologic cancers, including tubal and endometrial.

One theory holds that the genes governing differentiation during embryonic development re-emerge to shepherd the uncommitted cells to the cell type of their respective tumor. *HOX* genes, which encode for transcription factors that play pivotal roles in controlling developmental patterns along various axis's, are normally expressed uniformly along the müllerian duct early in embryonic development and are specific for location; *Hoxa9* in the primordia of the fallopian tube, *Hoxa10* in the developing uterus, *Hoxa11* in regions destined to become the lower uterine segment and cervix, and *Hoxa13* in the future upper vagina (18,19). These genes are not expressed in normal ovarian surface epithelium, but are expressed in ovarian carcinoma according to histologic differentiation (serous, endometrioid and mucinous).

Like other solid tumors, ovarian cancer is thought to result from a sequential accumulation of genetic changes in tumor suppressor genes and oncogenes. For example, mutations in p53 and c-Myc are commonly detected in serous carcinomas. (20,21) However, the particular early molecular and genetic events associated with neoplastic transformation of the ovarian surface (OSE) or ovarian cortical inclusion cyst epithelium are still largely unknown. Study of early changes has been hampered by the absence of a universally agreed upon candidate in the OSE.

Although the mechanisms for neoplastic transformation of the OSE have remained elusive, there is no question that the ovarian cortical epithelium plays a part in many ovarian tumors. First, cortical inclusions commonly harbor metaplastic change (22). Second, the consistent localization of mucinous, clear cell, endometrioid and low grade serous tumors to the ovary, strongly implies an origin in müllerian epithelium on the surface or the ovarian cortex. There are few epidemiologic studies of ovarian inclusion cysts but the limited data suggests an association with other classical ovarian cancer risk factors including older age, lower age at menarche, older age at first birth, oral contraceptive (inverse), and high body mass index. (23-25) Models of cortical inclusion formation include repair of ovulation sites, formation of fibrous or salpingeal adhesions or cortical invaginations of OSE with aging. The latter process leads to invaginations of the OSE into the ovarian cortex and the formation of cortical inclusions. The common element in cortical inclusion formation is the entrapment of OSE in a stroma-rich environment, and stroma may be the source of the metaplastic trigger. In addition, there is growing interest in the etiologic role of inflammation, which accompanies each ovulation, with an associated cytokine release, influx of inflammatory cells, and tissue reconstruction. This mechanism has been postulated to stress OSE cells such that they are predisposed to genetic damage, metaplasia, and malignant transformation. (9,26,27).

Epidemiologic studies demonstrate a decreased risk of epithelial ovarian cancer by factors that suppress ovulation, including pregnancy, lactation and oral contraceptives. (28,29) Further support for a causative role of ovulation in cancer comes from the observation that cancer of the OSE is rare in animal species that ovulate infrequently, whereas it is common in hens, which, like humans, are frequent ovulators. (30) However, ovulation cannot be the whole explanation and other triggers and cancer risk factors will be discussed later in this chapter. Inclusion cysts, while an attractive target for genetic mutations per se, may not be a mandatory precursor. Many high grade serous carcinomas likely arise either directly from the ovarian surface or the fallopian tube. Also, intraparenchymal tumors can arise in endometriotic cysts rather than cortical inclusions. In addition, some serous and mucinous tumors appear to arise from slow-growing surface neoplasms (adenofibroma or cystic borderline tumor) that are not linked to ovulation

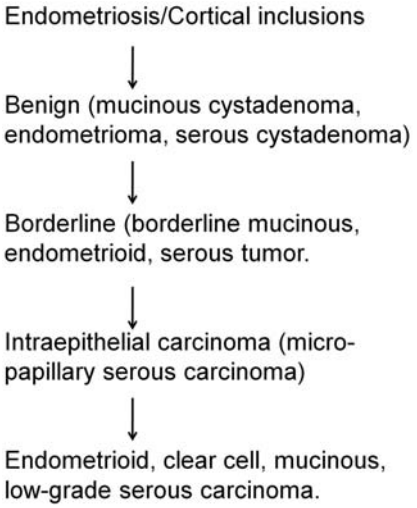

Figure 1. The "ovarian" pathway to epithelial malignancy. This pathway would be responsible for the more treatable forms of ovarian cancer and evolves gradually, with pre-existing benign conditions emerging from either cortical inclusions or endometriosis. Certain gene mutations, such as ras, raf, beta catenin and pTEN increase in frequency with severity of disease.

Irrespective of how Müllerian epithelium becomes incorporated into the ovary, the role that this epithelium plays in the evolution of ovarian cancer is inextricably linked to the molecular pathways proposed. One, traditionally designated the Type I pathway, encompasses endometrioid, mucinous and low-grade serous tumors, and involves mutations in the k-ras, b-raf, and pTEN genes, among others. These tumors appear to arise in morphologically visible pre-existing conditions, including endometriosis, cortical inclusions, adenofibromas, and in some instances tumors with both benign and malignant components (Figure 1). These tumors are also frequently confined to the ovary or are present within an ovarian cyst. The so-called Type II pathway has been reserved for high-grade Müllerian carcinomas, most notably high-grade serous carcinomas, and involves mutations in the p53 tumor suppressor gene. (31,32) This group of tumors is notorious for presenting in an advanced clinical stage; over 90% of serous carcinomas are discovered after involvement of the peritoneal surfaces has taken place, a good reason why efforts at early detection have failed to date. A fuller understanding of high-grade serous carcinomas carries three requirements. First, traditional assumptions regarding the origin of these tumors must be stripped away and their primary site re-assessed in light of more objective data. Second, a candidate non-neoplastic precursor lesion must be identified and linked to the malignancy. Third, other events leading to malignancy must be taken into account and used to expand the precursor model. A critical aspect of this exercise is to merge the second and third components into a cogent framework that is visible and reproducible at the pathologic level. This transition requires an understanding of three interrelated entities, serous tubal intraepithelial carcinomas (STICs), p53 signatures, and secretory outgrowths of the fallopian tube (SCOUTs).

3. CHANGING THE LANDSCAPE: SEROUS TUBAL INTRAEPITHELIAL CARCINOMA

3.1. The BRCA model and STICs

Beginning in the late 1990s, the fallopian tube began to emerge as an important source for pelvic serous carcinoma. (33) The emergence of this organ as an important participant was delayed for the simple reason that very few serous carcinomas were assigned to this site. In fact, tubal carcinoma was estimated to be nearly one-fiftieth as common as its ovarian counterpart. (34) Concurrent with this concept was a change in the perception of early serous carcinoma in BRCA+ women. As recently as 2003, Piek *et al* noted that in symptomatic BRCA+ women, nearly 90% of the tumors were classified as ovarian in origin. (35) However, as sporadic reports of early tubal carcinomas began to emerge, investigators recommended complete examination of both tubes and ovaries in women undergoing risk-reducing salpingo-oophorectomy. As these cases began to accumulate, the number of reported tubal primaries increased. (36−41) Women with BRCA1 and BRCA2 mutations carry a significant risk of pelvic serous carcinoma estimated as high as 50% (BRCA1) if followed indefinitely. The risk is highest for women over the age of 40 and currently, many of these women undergo risk-reducing surgery at this age or above when they become aware of their risk. The tubes and ovaries are typically sectioned at 2-mm intervals and scrutinized by the pathologists, who must exclude both invasive carcinoma and its non-invasive counterpart, serous tubal intraepithelial carcinoma (STIC). Similar to its counterpart in the endometrium, STIC carries a risk of spread to adjacent pelvic surfaces. The proportion of healthy women whose tubes or ovaries disclose a malignancy varies, but is approximately 8% based on several studies. Based on these studies and one by Cass *et al*, the origin of these early carcinomas is almost exclusively the distal fallopian tube (fimbria). (42) At Brigham and Women's Hospital, a modified protocol (SEE-FIM) has been instituted that amputates and sections the fimbria at 2-mm intervals in a longitudinal fashion. This protocol, used at our institution and others, has resulted in the detection of STICs, not only in BRCA+ women but in occasional women with a very low index of suspicion (40) (Figure 2).

3.2. STIC in the general population of ovarian cancers

Several studies have shown that STICS can be detected in from 35-50 percent of pelvic serous carcinomas. Kindelberger *et al* studied a range of tumors in women, almost all of which had no history of germ-line BRCA mutations. (43) They identified STIC in 20 of 43 (47%) tumors classified as ovarian in origin (Figure 2). Carlson *et al* identified STIC in 9 of 19 (47%) primary peritoneal serous carcinomas; (44) Roh *et al* identified STIC in approximately 35% of cases. (10) The conclusion from these studies was that a significant minority of high-grade serous carcinomas could be traced to the distal fallopian tube. Over 90% of serous carcinomas harbor mutations in p53, as do all of the STICS that we have analyzed to date (8,45). In all instances where both the STIC and remote tumors were analyzed, identical p53 mutations could be

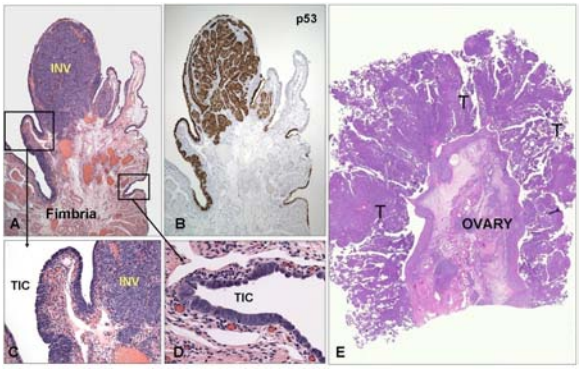

Figure 2. Serous tubal intraepithelial carcinoma (TIC) (left) associated with extensive ovarian cancer (right). T = ovarian tumor, INV – invasive tubal carcinoma, p53 = following immunostaining for p53. From reference 43, with permission.

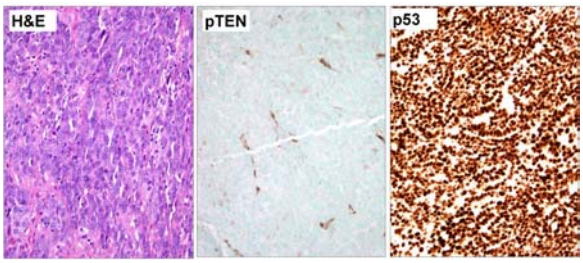

Figure 3. Gene mutations associated with ovarian and extra-ovarian pathways can co-exist in all high-grade pelvic mullerian carcinomas. In this endometrioid carcinoma that was presumed to arise in the ovary, expression of both pTEN and p53 are altered, the former by loss of staining relative to the normal epithelial and stromal cells (seen here) and the latter by intra-nuclear accumulation of mutated protein.

identified, linking the two processes. (8) This is strong evidence for a causal relationship between the two.

3.3. Unanswered questions

Two important questions remain to be sorted out concerning the above data. The first is the directionality of tumor spread in high grade serous carcinomas. There is no conclusive experimental evidence that tumors begin in the fimbria and spread to the peritoneal surfaces; however, the not infrequent detection of STICs in the distal tube of BRCA+ women in the absence of other disease is a strong endorsement of this possibility. The second is whether a higher percentage of BRCA+ serous carcinomas arise in the tubes vs those emerging in the general population. Because 85% of early carcinomas are documented in the distal tube, there is a temptation to assume that this high figure is unique to the BRCA+ subject. However, symptomatic BRCA+ women have a frequency of STIC – about 40%- that is similar to the general population. (46). This leaves open the possibility that BRCA+ women may be more prone to cancers arising in the ovary than is inferred from the population undergoing risk reducing salpingo-oophorectomy. This is an important question that must be addressed.

3.4. Re-visiting high-grade Müllerian carcinomas

There is increasing evidence that the high-grade category of müllerian adenocarcinoma is one that cannot be easily classified as to cell type or always reproduced, a function of both morphologic and molecular overlap. The prototypical high-grade neoplasm – high grade serous carcinoma – contains p53 mutations in virtually every instance. In contrast, high grade endometrioid carcinomas, which might be envisioned an extension of the endometrioid phenotype, are emerging as a neoplasm with many features in common with their serous counterparts. p53 mutations have been isolated in as many as 70% of these tumors, and the two tumor types share a high frequency of p53 and p16 immunostaining. (45,47) Staining for WT-1, typically assigned to serous carcinomas, is frequent albeit less so, in the high grade endometrioid carcinomas. This is similar to that observed in high-grade carcinomas of the endometrium, where both the endometrioid and serous subtypes share similar characteristics. The differences in the two tumors lie in their patterns of distribution. High grade endometrioid carcinomas of the endometrium have a different metastatic pattern. (48) Similarly, high-grade endometrioid carcinomas of the ovary are more likely to present as a single or dominant ovarian mass and to not be associated with a tubal intra-epithelial carcinoma. (10) The overlap in pathway disturbances extends to pTEN and PAX2 as well (Figure 3) (47).

4. EMERGING PRECURSOR CANDIDATES IN THE FALLOPIAN TUBE

4.1. The p53 signature

The fallopian tube mucosa is composed of two readily distinguishable cell types. The ciliated cells are presumed to be terminally differentiated cells that emerge from a non-ciliated population, conveniently termed the secretory cells. There is experimental evidence - albeit limited - that this transition occurs under the influence of estrogen. Thus, in reproductive age women, the tubal lining is composed of alternating clusters of both cell types.

During their initial study of the tubal mucosa in BRCA+ women, Medeiros *et al.* noted stretches of secretory-type cells exhibiting strong p53 immunoreactivity but appearing non-proliferative and histologically benign (40). The observed loss of ciliated cells was reminiscent of "dysplastic" lesions earlier described by Piek *et al.* (49). This group of investigators noted that these changes occurred in secretory cells, for which Bcl2 or HMFG2 are markers (8,49). Lee *et al* termed these stretches of strong p53 staining 'p53 signatures' (8). Such lesions have been detected by immunohistochemistry in histologically normal tubal epithelium (Figure 4). P53 signatures are characterized by intense p53 immunostaining and mutations in the p53 gene (8). p53 signatures in the distal tube can be found in up to 50% of salpingectomies of all women and are equally common in non-neoplastic tubes from BRCA mutation carriers and controls, but more

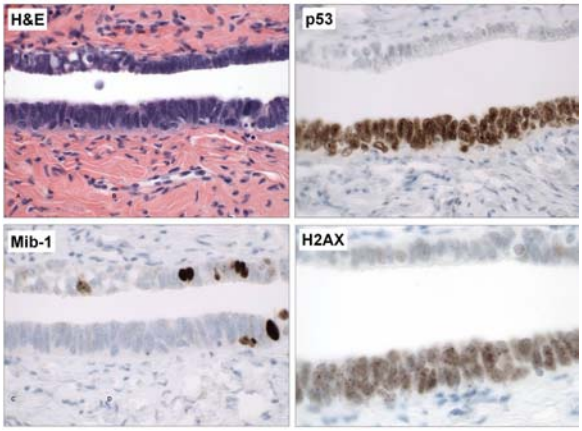

Figure 4. p53 signatures are characterized by bland appearing stretches of secretory cells with intense immunostaining for p53 without an increase in proliferation (Mib-1). Punctate staining for H2AX signifies activation of the DNA damage response.

frequently present and multifocal in fallopian tubes that also contain STICs where the two lesions can be shown to share a common p53 mutation (8) (Mehra K, Crum C, unpublished). These findings may have broad implications for both sites of origin and management of women at risk. Lee *et al*. discovered that p53 signatures stain strongly for the DNA damage marker γ-H2AX. γ -H2AX is a phosphorylated form of the histone H2AX (8). Phosphorylation of H2AX by the DNA damage-sensing kinases ATM and ATR occurs rapidly at sites of DNA double strand breaks (50). The presence of 'p53 signatures' in the fimbria of normal women provides the first evidence that, under normal physiologic conditions, fimbrial epithelial cells experience genotoxic damage and trigger a DNA damage response. Based on these observations, Lee *et al.* hypothesized that p53 signatures could represent the elusive ovarian serous carcinoma precursor (8). To determine whether p53 signatures also occur in the ovarian epithelium, Folkins *et al.* examined the ovaries and fallopian tubes of 75 BRCA mutation carriers. They detected a total of 29 signatures in the tubal mucosa but only one in OSE and none in cortical inclusions (51), confirming that p53 signatures preferentially arise in fallopian tube epithelium rather than OSE.

The high frequency of p53 signatures in women both with and without genetic risk might seem paradoxical if this entity is to be assigned a role as a serous cancer precursor. Nevertheless, the multi-step model of carcinogenesis requires that multiple events take place before a malignancy can result. In this model, the p53 signature or any early event is by definition common, since only a subset of these precursor lesions will acquire the necessary and subsequent events to produce carcinoma. BRCA+ women are particularly susceptible to at least one subsequent event, presumably the inactivation of BRCA1 or BRCA2 which would be more likely in the face of a preexisting germ-line mutation.

p53 signatures are associated with lower parity and higher age at first childbirth similar to ovarian cancer. (52) Moreover, they are associated inversely with body mass index, similar to non-endometrioid (or serous) carcinomas of the ovary (23).

The mechanism for the expansion of secretory cells that forms the underpinning of early serous carcinogenesis remains to be fully resolved. First, it is not clear whether these expansions are invariably the result of a clonal event, although the finding of mutations in p53 strongly supports this. The consistent upregulation of HMGA2 in p53 signatures and serous carcinomas is further evidence that the former consists of a unique (clonal) cell population (53). Moreover, these secretory outgrowths that characterize the p53 signature are similar in appearance to the clonal events proposed for endometrioid carcinogenesis in the uterus that consist of discrete genetic disturbances (54). An equally compelling and unanswered question is whether the secretory cell outgrowths reflect an inability to undergo ciliation due to an interruption of the normal differentiation process. If this proves to be the case, the p53 signatures and similar entities could provide insights into both the origin and the replicative/differentiation capabilities of non-ciliated cells of the tube, teasing apart the secretory and stem cell properties and providing a clearer picture of their role in the occasional development of malignancies. (55)

Because the p53 signature is common in women with and without inherited mutations in BRCA1 or 2, it is presumed that loss of BRCA1 or 2 functions is not necessary for its formation (51, 56). This is in contrast to serous carcinomas which are commonly associated with loss of heterozygosity of the gene (LOH) in BRCA+ women and loss of function in nearly one half in sporadic cases (57). In the latter scenario, BRCA1 or BRCA2 null cells survive if there is a concurrent loss of p53 function, greatly increasing the odds of a malignancy (58). In the BRCA+ individual, this important step - loss of BRCA1 or 2 function - is heightened by the pre-existing haplo insufficient state. A graphic illustration of this requirement is seen in tubes from women with Li Fraumeni syndrome, who have an inherited mutation in one of the *p53* alleles (59). We analyzed the distal fallopian tubes from three cases of LFS and discovered a strikingly high frequency of p53 signatures in this site (60). Fallopian tubes from LFS display modest p53 immunostaining, not unlike that in the general population. The extensive number of p53 signatures appears to signify LOH of the corresponding allele with inactivation of the gene and accumulation of the residual mutated allele leading to strong nuclear staining (Figure 5). The high frequency in the distal tube can be interpreted as due to both the susceptibility of this site and the relatively lower degree of "genotoxic stress" required to produce a single (in this case second) "hit" on the gene relative to women with wild type p53. Why LFS is not associated with pelvic cancer is unclear, but could be explained by the fact that despite the abundance of p53 signatures, inactivation of a second critical gene – such as BRCA – is no more likely that that of the general population. This underscores an important tenet in carcinogenesis, which is both that multiple steps are required and that the absence (or prevention) of any one step could significantly reduce cancer risk (6)

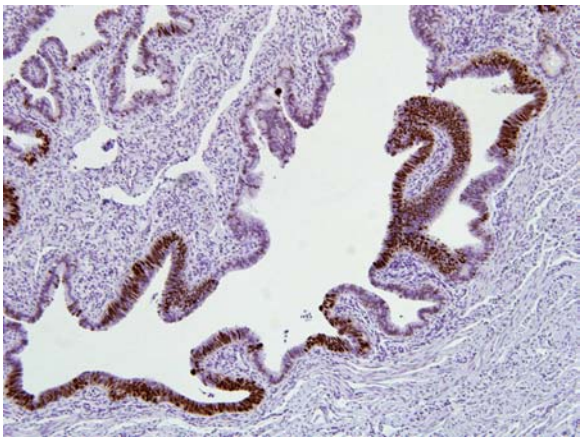

Figure 5. A distal fallopian tube from a woman with Li Fraumeni Syndrome is immunostained for p53, revealing multiple p53 signatures.

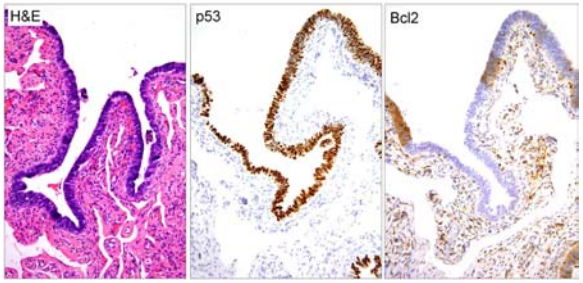

Figure 6. Functional loss of other genes in a p53 signature signifies the potential extent of genetic damage. Here a p53 signature from a BRCA+ woman contains discrete foci in which bcl-2 expression has abruptly disappeared.

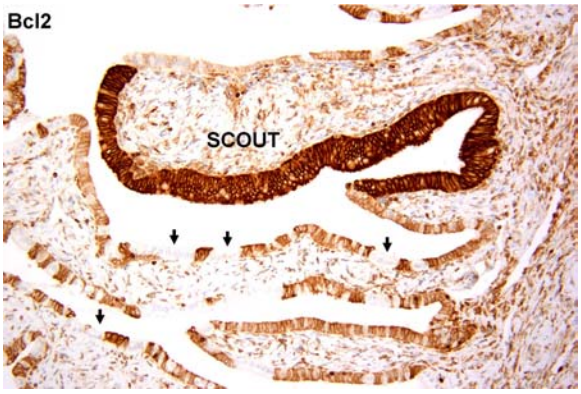

Figure 7. A secretory cell outgrowth (SCOUT) in the distal tube. Bcl2 staining of the the adjacent normal epithelium displays a heterogeneous population of secretory (staining) and ciliated (non-staining) cells. Strong consecutive and homogeneous staining for Bcl2 highlights a SCOUT.

4.2. Secretory cell outgrowths (SCOUTs): more pathways coming into play?

Because the pathway to malignancy is multi-genic, it is reasonable to assume that additional events take place in the fallopian tube mucosa. Moreover, the predilection of both p53 mutations and DNA damage for the secretory cell population is well established. Thus, it is likely that other gene perturbations occur either in concert with p53 signatures or separately. One example is the loss of expression of certain genes, such as Bcl2 (Figure 6), which we have witnessed in a significant minority of p53 signatures. Another example, and one that we have just begun to study, is the existence of other secretory cell outgrowths, or SCOUTs, that occur in the absence of abnormalities in p53 staining (61). These SCOUTs are unique both by the absence of p53 mutations and by the fact that a variable degree of ciliated differentiation can occur.

In general, the lining epithelium of the fallopian tube is characterized by pseudo-stratified ciliated and secretory cells that alternate along the mucosal surface. It is generally assumed that the "secretory" cell is a precursor to ciliated cells, undergoing ciliation under hormonal control, as suggested by *in vitro* studies (62). Whether one or multiple types of non-ciliated cells are involved is not clear but a plausible scenario is one in which ciliated cell differentiation is triggered by certain factors. Thus some SCOUTs, particularly those associated with p53 mutatinons exhibit minimal ciliation while other SCOUTs not associated with p53 mutations exhibit variable ciliation (Figure 7). Either process would appear as an aberration against the background of this mixed population. Given that loss of p53 function defines one subset of SCOUT, it is reasonable to assume that SCOUTs signify the alteration of additional genes. Thus, SCOUTs signify a variable ability to undergo ciliation on one hand and the expansion of a specific basal cell population on another (Figure 7). What is intriguing is that SCOUTs seem to be a discrete entity, like the p53 signatures, and preliminary evidence suggests that SCOUTs, like p53 signatures, are more commonly associated with pelvic serous carcinoma. (61) We are currently analyzing SCOUTs for loss of function in a range of genes, the intent being to expand the gene signature characterizing the early events in tubal carcinogenesis (Figure 8). Candidates include not only p53, but also PTEN and PAX2 (61).

5. FUTURE DIRECTIONS

It is becoming increasingly clear that there is wide gulf between the molecular events associated with advanced ovarian cancer and the early steps of serous carcinogenesis. The genomic aberrations that typify advanced pelvic cancers consist of variations in copy number, gene amplifications and upregulation of numerous genes, many exploited with relatively little success in the effort to detect serous cancer at an early stage. The overwhelming emphasis on advanced malignancy is understandable, given both their conspicuous threat and the relative ease with which they can be manipulated and studied in the laboratory. In contrast, precursor lesions are small, difficult to work with and do not lend themselves to projects that can easily be funded. That said, the following initiatives are in order

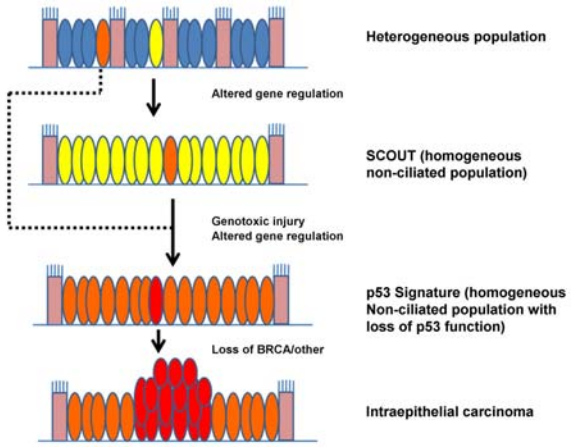

Figure 8. An expanded model for serous carcinogenesis. Secretory (elliptical) cells in normal mucosa (I) are subject to independent genetic and epigenetic events (II), leading to focal secretory cell outgrowths with variable ciliation (yellow; III) including p53 signatures in the distal tube (orange). In the latter, additional events may lead to a TIC (red) (IV). Acquisition of additional and undisclosed genetic events (?, blue) confers the metastasizing phenotype

A complete profile of SCOUTs and p53 signatures is needed to uncover the number of genetic alterations involved in early serous carcinogenesis and their trigger(s). It is highly plausible that once a particular threshold is crossed, the loss of cell cycle control coincides with substantial genomic instability. Understanding the genetic events leading up to this critical juncture is important.

It is entirely possible that the risk factors for one precursor event may not be the same as for others. For example, inactivation of one gene could entail a hormonal-related event while another could be exposure to a genotoxic event, such as ovulation. Epigenetic (methylation) and genetic alterations could work together. Work by Windschwendter and colleagues has raised the tantalizing possibility that epigenetic alterations in gene function can be identified not only in ovarian tumors but in remote sites such as the endometrium (or fallopian tube) (63,64). Teasing apart these mechanisms might reveal more than one set of risk factors operating at different points in space or time.

Successful interventions tailored to a single component of the precursor cascade conceivably could prevent the endpoint – malignancy or reduce its incidence. For example, oral contraceptives (OCPs) are assumed to prevent ovarian cancer via suppression of ovulation. However, other scenarios are plausible; OCPs could suppress other critical pathway disturbances and might be improved upon and tailored to address these critical variables.

It is mandatory that research be devoted to the pathogenesis of this surprisingly lethal disease, and it will require stripping some assumptions and testing new concepts in the framework of a visible carcinogenic pathway. The vistas uncovered will no doubt reveal major challenges, but until these challenges are recognized and faced, the collective creativity of the field of ovarian cancer research will not be fully tested.

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