Chemoresistance, Dormancy and Recurrence in Platinum Drug Therapy of Ovarian Cancers

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Abstract

Ovarian cancer is the most deadly of gynecological malignancies. Lineage analyses have suggested broadly classifying ovarian cancers into two types: Type I, which includes low grade cancers with intact TP53, and Type II, which comprises high grade cancers with defective TP53. If detected in early stages, surgical resection of ovarian tumors results in a high rate of long-term survival; however, most ovarian cancers are detected at advanced stages. The standard first line treatment for advanced stage ovarian cancer is maximal surgical cytoreduction followed by platinum-based combination chemotherapy. Although the overall prognosis for less aggressive Type I ovarian cancers is better, their response to chemotherapy is generally weaker than that of the Type II ovarian cancers. Despite an initially favorable response of optimally debulked Type II ovarian cancers to platinum-based chemotherapy, the rate of recurrence is high, making the long-term survival rate quite poor. The dynamics of the response of high grade ovarian cancers platinum suggest that the tumors are phenotypically heterogeneous, and that a subpopulation of tumor cells is relatively resistant to chemotherapy. The resistant tumor cell population persists after chemotherapy in a state of dormancy, with recurrent tumors arising upon transformation of the dormant cells back to malignant growth. This chapter will consider

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how lineage, histological subtype, and grade influence the differential responses of ovarian cancers to platinum-based chemotherapy. In addition, mechanisms contributing ovarian cancer resistance to platinum drugs and to tumor cell entry into and exit from dormancy will be discussed.

Introduction

Developmental Origins and Anatomy of the Ovaries

An understanding of the pathogenesis of ovarian cancer requires an appreciation of the embryologic development and anatomy of the ovaries. The ovary initially takes form as an 'indifferent gonad,' which first becomes apparent as a thickening of the intermediate mesoderm along the dorsal body wall, termed the urogenital ridge. The urogenital ridge eventually becomes two distinct structures, a gonadal ridge and a mesonephros. Some cells of mesonephric origin remain joined to the gonadal ridge, and this connecting remnant is the mesovarium. The gonadal ridge mass differentiates into a central part, the ovarian medulla, and a covering of a surface layer of flat to cuboidal, single layered cells called the germinal epithelium. Local steroid hormone action and other parenchymal microenvironmental cues are postulated to be involved in gonadal ridge differentiation. While the ovarian surface epithelial cells appears morphologically identical to other mesothelial cells lining the celomic cavity and shares common surface microenvironment by virtue of facing the abdominopelvic cavity, the ovarian surface epithelium is distinctive in being the only derivative of celomic mesothelium that does not express the marker CA125. This surface glycoprotein of unknown function is, in the adult an epithelial differentiation marker and tumor marker for ovarian and Mullerian duct-derived tumors. It is expressed by epithelial cells of the fallopian tube, endometrium and endocervix, and also by mesothelial cells lining the visceral and parietal peritoneum, pericardium and pleura. Its lack of expression in ovarian surface epithelium suggests that this epithelium may be subject to specific local microenvironmental influences that render it less committed to mature mesothelial phenotypic differentiation when compared to the rest of the pelvic peritoneal lining cells. However, CA125 expression re-emerges in a number of ovarian carcinomas, suggesting plasticity of the ovarian surface epithelium, at least under pathologic conditions.

Between the ovarian medulla and the surface epithelium, a number of ova are found. The immature ova originate from cells from the dorsal endoderm of the yolk sac. These cells migrate from near the allantois, along the hindgut to the gonadal ridge, where they undergo mitosis to become diploid stem cells called oogonia. In colonizing the primordial gonads, the oogonia migrate into the germinal epithelium, and are carried into the underlying stroma by bud-like ingrowths. The oogonia become surrounded by a layer of connective tissue cells, forming rudimentary follicles. The origin of the granulosa cells is still controversial. It is possible that mesonephric cells closely associated with the oogonia proliferate throughout development to form the granulosa cells, or that the granulosa cells develop from cells from the surface epithelium of the ovary and break apart into cell clusters that undergo follicular development. A tunica albuginea develops between the surface germinal epithelium and the ovarian medulla. The ovaries are formed in the abdominal cavity, and then descend into the pelvic cavity. This involves the gubernaculum, a fibrous tissue band that runs from the abdominal wall to the fundus of the uterus and limits descent of the ovary to the appropriate level. The portion of the gubernaculum that lies between the ovary and the uterus becomes the ovarian ligament.

Concurrently with the development of the ovaries, the celomic epithelium in the vicinity of the gonads invaginates to give rise to the left and right paramesonephric, or Mullerian ducts. The Mullerian ducts differentiate to eventually form the fallopian tubes, uterus and upper vagina. Thus, the perigonadal celomic epithelium represents an embryonic field with the ability to differentiate along multiple different pathways, which

include the mucosal epithelium of the fallopian tubes, endometrium, and endocervix. The close development of ovarian germinal epithelium and Mullerian epithelium are noteworthy for our discussion of ovarian and peritoneal surface carcinogenesis, specifically in relation to the 'field effect' hypothesis. This will be explicated further below.

Anatomically, the ovaries are paired, almond-shaped organs which lie within the pelvic cavity on either side of the uterus, at the level of the bifurcation of the common iliac artery. Each ovary is attached to the uterus by an ovarian ligament, and to the pelvic side wall by a suspensory ligament which additionally houses the ovarian blood and lymphatic supply. The ovary lies in an ovarian fossa, or shallow depression in the posterior part of the broad ligament of the uterus. As previously mentioned, the ovary is unique in the abdominopelvis in not being covered by the peritoneum that invests all other organs. The ovarian surface lining, a modified peritoneum called the germinal epithelium, is in continuity with the visceral peritoneum covering adjacent organs. The absence of a peritoneal covering is to allow an egg cell after to gain access after ovulation to the infundibulum of the closely apposed fallopian tube. It is this association that is implicated among the various pathways of ovarian carcinogenesis.

Risk Factors for Ovarian Cancer

Cancer is not a new disease. As early as 3000–1500 B.C. in ancient Egypt, there were documented descriptions of cancers of the breast, among other tumors, and the disease was attributed to acts of the gods. In the fifth century B.C., Hippocrates broke from tradition by postulating that cancers are due to natural causes, specifically resulting from imbalances in bodily 'humors'. A further development occurred in the Middle Ages, when identification of families and villages with high incidence of cancer brought forth the concept that cancer may be due to either inherited or environmental causes. A new frontier was entered when Rudolf Virchow examined tumors under the microscope, and made the observation that "every cell is born from another cell." He established cancer as a cellular disease. Twentieth- and twenty-first century advances in cancer biology are largely the result of developments in the field of molecular genetics. Indeed, cancer today is considered a genetic and epigenetic disorder, with the identification of its association with disease-specific gene mutations and heritable defects in chromatin organization and modification.

While ovarian cancer is rare, it is the most common cause of death from gynecologic malignancy in women in the United States, and is the fifth leading cause of cancer deaths among American women. Despite its lethality, the etiology of ovarian cancer is poorly understood. Family history is the most important and best defined risk factor to date, although it accounts for only 5–10% of cases. Women with two or more affected relatives or a relative diagnosed under 50 years of age, have the highest risk. There are three types of hereditary ovarian cancer syndromes with autosomal dominance:

(i) Hereditary breast/ovarian cancer syndrome, which shows the highest risks (up to 50%) for women with family histories of breast or ovarian cancer and mutations in the BRCA1 and BRCA 2 genes. The BRCA1 gene is present in two copies, one located on paternal chromosome 17, and the other on maternal chromosome 17. The BRCA2 gene is similarly present in two copies, one paternal and the other maternal, located on each chromosome 13. An individual who inherits one mutated copy of either of these genes from a parent, may never develop cancer but has an increased risk of up to ~50%, if the other chromosomal copy of the gene acquires mutation and, additional mutational events occur.

(ii) Lynch Syndrome II or hereditary nonpolyposis colon cancer/ovarian cancer, refers to those ovarian cancers which occur in families with a high incidence of cancers of the colon and endometrium. It is associated with mutations in DNA mismatch repair genes: hMSH1, hMSH2, hPMS1, and hPMS2.

(iii) Hereditary site specific ovarian cancer refers to cases for which there is a family history

of ovarian cancer but no specific gene has been identified. Some of these cases may not be a direct result of a gene mutation, but rather of mutation resulting from group exposure to a specific environmental carcinogen by virtue of a common habitat or other shared practices.

There are other, less strongly associated risk factors for ovarian cancer. Endometriosis has a rare, roughly 1% incidence of malignization which, when it occurs results in the development usually of either endometrioid or clear cell carcinomas. Co-existing with endometriosis, infertility is also considered a risk factor for ovarian cancer. Early menarche and late menopause are weakly associated risks. Factors considered to decrease a woman's risk for ovarian cancer include: pregnancy (inverse relationship, with more pregnancies conferring greater protection), breastfeeding, and oral contraceptive use (inversely related, with longer use conferring greater protection). These reproductive, menstrual and hormonal factors have been associated with lifetime number of ovulations, which are felt to be directly related to ovarian cancer risk. It has been postulated that the disruption of the ovarian surface epithelium is the inciting event for development of cancer, particularly in the case of high-grade serous carcinomas. More specifically, the damage sustained by the surface tissue during ovulation, and the resulting repair process and inflammation that necessarily ensue, provide the opportunity for genetic aberrations including mutations that may eventually result in cancer. Conversely, decreased ovarian function resulting from surgical factors such as hysterectomy or fallopian tubal ligation, which aore thought to lead to partial devascularization and fewer ovulations, are protective and have been found to be associated with decreased ovarian cancer risk. Other risk factors that have been proposed in the past, but have not been proven to be of significant risk, include: talc exposure to diaper area in female infants, dietary fat, smoking, obesity, use of menopausal hormones, alcohol, caffeine, and environmental and occupational risk factors.

Histopathogenesis of Ovarian Cancer

Current thinking is that ovarian epithelial cancer arises in one of two ways: (1) by de novo transformation without an identifiable precursor lesion, believed to occur in the development of high-grade serous carcinomas; or, (2) by a stepwise progression from hyperplasia to adenoma, to borderline tumor to cancer, believed to occur in the development of low-grade serous carcinomas, mucinous carcinomas, and endometrioid and clear cell carcinomas. Most endometrioid and clear cell carcinomas are believed to arise through a stepwise series of transformations, and many of these tumors are found with co-existing endometriosis which, though largely a benign condition, is thought to be a precursor. Only a small fraction of endometriosis cases, less than 1%, undergo malignant transformation. In many endometriosis-associated cancers, the diagnostic pathologist is able to find associated hyperplasia, adenofibroma, or borderline malignant areas which are suggestive of an antecedent process. In contrast high-grade serous carcinomas, previously thought to occur via this transformation spectrum, are currently believed to arise de novo from the surface epithelium of the ovary or, in some cases from the fallopian tube mucosal epithelium. Molecular biological evidence supports this dualistic model of ovarian carcinogenesis: the genesis of high grade serous carcinomas appears to be driven by early mutations that promote genomic instability, in particular TP53 mutations (Shih Ie and Kurman 2004), whereas low-grade serous carcinomas exhibit mutations in K-ras and BRAF genes, endometrioid tumors harbor pTEN gene mutations, and mucinous tumors show mutations in the K-ras gene, all in the context of nominal TP53.

Recently, it has been proposed that many high-grade serous carcinomas of the ovary or peritoneal surface may actually arise from the mucosa of the fimbriated end of the fallopian tube. Lee et al. (2007) found eight cases of ovarian cancer with co-existing in situ carcinoma of the fallopian tube mucosa, and identified the same TP53 gene mutations in both sites. Further,

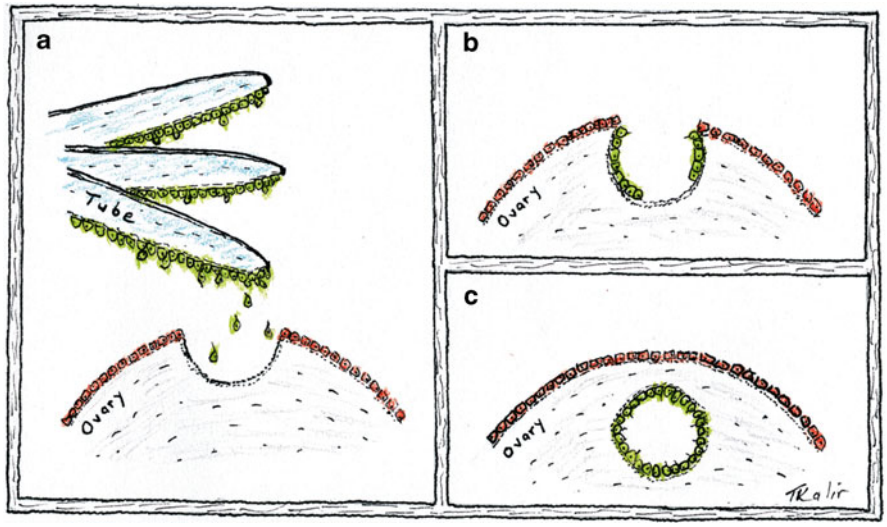

Fig. 7.1 *Panel A* shows exfoliation of some fallopian tube mucosal epithelial cells onto the surface of the ovary in an area disrupted by a recent ovulation. *Panel B* shows the proliferation of the fallopian tube epithelial cells along the disrupted ovarian focus. *Panel C* shows that, under the influence of proliferating ovarian surface epithelial cells causing reepithelialization of the ovarian surface and possible epithelial-to-mesenchymal cell conversion inducing formation of a matrix, the fallopian tube cells become walled off, forming a cystic 'inclusion'

Kindelberger et al. (2007) showed that in cases of primary peritoneal surface carcinoma, more than half of the fallopian tubes displayed mucosal involvement, often with in situ carcinoma. In a series study of risk-reducing salpingooophorectomy specimens from women with BRCA gene mutations, Medeiros et al. (2006) found clusters of TP53 defective cells and intraepithelial carcinomas in the mucosa of the fallopian tubes of roughly 30% of the women, while these changes were not present on the ovarian surface. Crum et al. (2007) studied prophylactic salpingooophorectomies of high-risk patients, and found that for incidental cancers, more than half occurred in the distal fallopian tube, supporting the idea that the distal fallopian tube is the site of origin of the majority of ovarian/fallopian tube cancers in high-risk women. Figure 7.1 illustrates the process, and the mechanism involved is as follows: After ovulation and prior to repair of the disrupted ovarian surface focus, the fimbriated end of the fallopian tube which is closely apposed to the ovarian surface, may exfoliate cells from its mucosal epithelium that implant in the unrepaired, disrupted site on the ovarian surface. As a result of ovarian surface germinal epithelial cell proliferation and synthesis of connective tissue type components of the extracellular matrix, the implanted fallopian tube epithelial cells also proliferate and become walled off from the native ovarian parenchyma, forming a cystic epithelial inclusion. Notwithstanding epigenetic and genetic changes that could impede this process from occurring, surface germinal epithelial cells could also contribute to formation of epithelial inclusions of ovarian surface epithelial origin, and these may subsequently undergo metaplastic change to resemble tubal epithelial cells. Hence, it remains unclear whether intraovarian epithelial inclusions which morphologically resemble fallopian tube cells, are truly of tubal origin.

It is noteworthy that high-grade serous carcinomas comprise the vast majority of ovarian cancers (roughly 80%). These tumors are quite unlike the simple, low-cuboidal normal ovarian surface epithelial cells, and in fact more closely resemble Mullerian-duct epithelia. Specifically, high-grade serous carcinomas of the ovary, fallopian

tube, endometrium and endocervix appear histologically and phenotypically indistinguishable. This supports the hypothesis of the tubal origin of at least a subset ovarian cancers. Because most patients with high-grade serous carcinomas present with advanced stage disease and bulky tumors involving multiple organs, the decision as to whether a tumor is fallopian tubal or ovarian in origin can, for a small subset of cases, be a daunting task for the diagnostic pathologist. Because the gross and microscopic appearances, and the patterns of spread are similar for these two entities, the determination of the organ of origin may at times be arbitrary. Historically, in the absence of other helpful findings, the organ with the greatest amount of tumor, usually the ovary, was considered the primary. However, Crum et al. (2007) have recently challenged this convention by proposing that fallopian tube fimbrial cancers may exfoliate cells which disseminate to the peritoneum, and that secondary sites of deposition may be larger than the primary.

The sequential histological changes that precede development of fallopian tube carcinoma have not been well elucidated. Fallopian tube mucosal epithelial hyperplasia, demonstrated by a proliferation of the pseudostratified columnar cells in the absence of marked cytologic atypia and mitotic activity, has been \traditionally considered to a variant of normal and not precancerous. Its incidence is fairly low. Fallopian tube preinvasive carcinoma (dysplasia or carcinoma in situ) was a rare diagnosis until the advent of risk-reducing salpingooophorectomy for women with BRCA gene mutations (Medeiros et al. 2006). The diagnosis of in situ carcinoma of the fallopian tube requires the presence of marked cytologic atypia, multilayered epithelium containing cells with large, pleomorphic nuclei and prominent nucleoli, and interspersed mitotic activity.

Multifocal carcinomas that involve the fallopian tube show three patterns: (1) synchronously detected multifocal carcinomas within the fallopian tube or tubes; (2) multifocal carcinoma involving various genital organs including the fallopian tube or tubes; and (3) direct spread of cancer, frequently intraepithelial carcinoma along the mucosa of the cervix and endometrium, to the fallopian tube. Roughly 20% of patients with fallopian tube cancer have bilateral involvement. It is not clear whether these additional foci of carcinoma represent synchronous tumors or metastasis. Woodruff et al. (1985) have proposed that multifocal disease may reflect neoplastic transformation of the common embryologic field, which includes the coelomic epithelium that covers the ovary, fallopian tube, and other pelvic peritoneum. The idea of a so-called field effect was first proposed by Slaughter et al. (1953) in his 'field cancerization' hypothesis, put forth to explain the development of multiple primary tumors and locally recurrent cancer. Initially a stem cell acquires genetic alterations and forms a patch, or clonal unit of altered daughter cells. Such patches can be recognized by TP53 mutations. The patch is converted into an expanding field, after additional genetic alterations are acquired and the cell displays a growth advantage over its neighbors; its proliferative field eventually displacing neighboring normal mucosal epithelial cells. Also in favor of this hypothesis is the observation that noncontiguous endometrioid carcinomas are not infrequently found simultaneously in the endometrium, ovary and fallopian tube mucosa. The frequent finding in these cases of associated endometriosis has implicated it as the site of multifocal neoplastic 'field' transformation. However, the debate remains unsettled as to whether these are multiple primary tumors or a single primary with metastases.

Ovarian cancers have a characteristic tendency to spread via the mechanism of exfoliation into the peritoneal cavity. Exfoliated cells are propelled by the normal circulation of the peritoneal fluid upwards along the right paracolic gutter to the undersurface of the right hemidiaphragm. Here they may implant and grow as surface nodules. The omentum is also a favored site of involvement, although all intraperitoneal surfaces are at risk. Exfoliation and implantation are one of two primary modes of spread of ovarian cancer. The other mode is lymphatic. Ovarian cancer cells metastasize via the retroperitoneal lymphatic spaces that drain the ovary. These follow the ovarian blood supply in the infundibulopelvic ligament and terminate in aortic and vena caval

lymph nodes, up to the level of the renal vessels. Lymphatic channels also pass laterally, through the broad ligament of the uterus and parametrial channels alongside the uterus, to terminate in the pelvic sidewall lymphatics, including the external iliac, obturator, and hypogastric chains. Spreading may also occur along the round ligament of the uterus, with resultant involvement of the inguinal lymphatics. Lymph node metastases are correlated with stage of the disease, and retroperitoneal lymph node involvement is found in a number of cases of advanced ovarian cancer.

Platinum Chemotherapy of Ovarian Cancer

Mechanisms of Platinum Cytotoxicity

Platinum drugs (carboplatin and cisplatin) have been employed with surgical debulking as adjuvant chemotherapy in combination with taxanes (docetaxel or paclitaxel) or cyclophosphamide for advanced ovarian cancers, and combinations with taxanes have been shown to be superior as a first line treatment (Piccart et al. 2000). Platinum chemotherapeutic reagents such as cisplatin form mostly intrastrand and some interstrand DNA adducts by crosslinking N7 positions of purines. Three intrastrand crosslink (1,2-d(GpG), 1,2-d(ApG) and 1,3d(GpNpG)) comprise 90% of the DNA lesions generated by cisplatin, while interstrand crosslinks comprise around 5% (Eastman 1986). The intrastand crosslinks are repaired by nucleotide excision repair (NER), whereas interstrand crosslinks (ICLs) are repaired by ICL repair, which is less well understood. Mechanisms of ICL repair have been proposed in which mammalian cells use novel excision repair reactions (requiring the XPF and ERCC1 proteins) to uncouple the crosslink (McHugh et al. 2001). The abundance of unrepaired interstrand crosslinks may be more closely associated with induction of apoptosis in cancer cells by cisplatin than the abundance of intrastrand crosslinks. Defects in ERCC1 (Damia et al. 1996) or low expression of ECRR1-XPF endonuclease (Arora et al. 2010), the latter of which is observed in metastatic testicular germ cell tumors, renders cancer cells exquisitely sensitive to cisplatin (Usanova et al. 2010).

The presence of platinum drug DNA adducts blocks both the progression of DNA replication forks and transcription, resulting in the activation of ATR; ineffective repair of crosslinks can lead to double strand breaks, resulting in activation of ATM signaling pathways. DNA replication forks stalled by intranuclear or internuclear crosslinks recruit ATR through ATRIP, resulting in assembly with TopBP1 and the 9-1-1 (Rad9, Rad1, Hus1) complex (Yan and Michael 2009). Depending on the cellular context, activated ATR kinase will phosphorylate several proteins, including effectors of cell cycle arrest (e g., H2AX, p53, Chk1), DNA repair (BRCA1/2), and apoptosis (p53, FANCJ). An early event in the response to DNA damage is the phosphorylation of H2AX by ATR, giving rise to intranuclear foci of phosphorylated histone H2AX. Accumulation of phosphorylated H2AX foci occurs within 4 hours after exposure to cisplatin, and depending on the dose and duration of cisplatin exposure, apoptosis is observed 18 or more hours later (Manju et al. 2006). Poly (ADP-ribose) polymerase (PARP-1) cleavage has been shown in some cancer cells to occur between 16 and 24 h after treatment with cisplatin (Manju et al. 2006). This lag time is an important characteristic of cisplatin-induced cell death. The events that precipitate apoptosis in ovarian carcinoma cells after exposure to cisplatin are not completely understood, but cytotoxic sensitivity to cisplatin is maintained, and possibly enhanced, in the face of defects in TP53 that are characteristic of the high grade forms of ovarian cancer. Implicated in the cytotoxic response of ovarian carcinoma cells to cisplatin is activation of ERK, and long-term sustained activation of SAPK/JNK and p38 kinases, followed by downstream induction of AP-1 and FAS-L expression (Mansouri et al. 2003). Enhanced accumulation of Fas-L and its binding to Fas (receptor) leads to activation of caspase 8, downstream activation of other caspases, and activation of mitochondrial death pathways (Muzio et al. 1998). Cells with defects in nucleotide excision repair cross complementing genes (ERCC1-4) display cytotoxic hypersensitivity to

cisplatin, suggesting that accumulation of DNA lesions also plays a role in the induction of cell death (Damia et al. 1996). Some studies have indicated that cisplatin lesions in DNA directly block transcription, leading to apoptosis (Ljungman and Lane 2004).

Molecular Lineages in Ovarian Cancer: Impact on Tumor Behavior and Outcome of Platinum Chemotherapy

As with most solid tumors, the majority of ovarian cancers and their metastasis have clonal origins (Khalique et al. 2009). A clonal origin for the tumor does not necessarily indicate that all the tumor cells are genetically identical, as intratumor genetic heterogeneity arises during tumor development (Khalique et al. 2007). In addition, epigenetic mechanisms can contribute phenotypic variability within the tumor cell population, including overt changes in cellular morphology of subpopulations of tumor cells, such as epithelial to mesenchymal transitions (Wu et al. 2012). Ovarian cancers differentiate into histologically distinct subtypes that resemble normal tissues: serous, endometrioid, and mucinous ovarian cancers resemble cells that line the glandular fallopian tube, endometrium, and endocervix, respectively. Clear cell ovarian cancers resemble cells found in nests in the vagina, and this histological relationship is suggested by the development of vaginal clear cell adenocarcinoma in daughters whose mother took diethylstilbestrol during pregnancy (Laronda et al. 2012). A slew of rarer histological subtypes, such as mixed and transitional, also appear as ovarian cancers. Within histological subtypes, macro-architecture also may impact tumor clinicopathological behavior (Veras et al. 2009).

The resemblance between histological subtypes of ovarian cancers and normal tissues is more than a coincidence. Patterns of gene expression in different histological subtypes of ovarian cancer correlate with those in the normal tissues they resemble. Colinear expression of *Hoxa9* (follapian tube primordia), *Hoxa10* (developing uterus) and *Hoxa11* (low uterine and cervical primarodia) genes is observed in Mullerian duct development in the mouse embryo (Taylor et al. 1997), but these genes are not normally expressed in adult epithelial cells lining the ovaries or inclusion cysts (Naora 2005). Expression of *HOXA9*, *HOXA10* or *HOXA11* are observed in serous, endometrioid, and mucinous ovarian cancers respectively (Cheng et al. 2005), indicating that histological phenotypes are driven by aberrant activation of normal developmental pathways. Over 90% of ovarian cancers are thought to arise from the epithelium that lines the surface of the ovary, ovarian inclusion cysts, or the fimbriae of the fallopian tubes, and are referred to collectively as epithelial ovarian cancers. This epithelium has been described as coelomic, mesothelial or transitional, but gene expression profiling studies have suggested that it is multipotent, expressing genes that maintain stem cell characteristics (Bowen et al. 2009). As such, these cells would have the potential to give rise to neoplasias with different histological subtypes, and although neoplastic ovarian cells lose expression of some stem cell genes, why they develop into and sustain specific histological subtypes is not understood. As will be discussed below, a fraction of the tumor cells may maintain a stem cell phenotype (cancer stem cell), and in contrast to the larger mass of histologically differentiated cells, this population may be responsible for tumor growth and recurrence after therapy.

Although ovarian cancer is often viewed clinically as one disease, histological (or morphological) subtypes of ovarian cancer can exhibit different behaviors, prognoses and responses to therapeutic intervention. The standard treatment for epithelial ovarian cancers is surgical debulking and chemotherapy with platinum analogues plus paclitaxel (Piccart et al. 2000). In contrast to advanced ovarian carcinomas displaying serous and endometrioid histology, advanced stage ovarian carcinomas displaying clear cell and/or mucinous histologies have been associated with shorter progression-free intervals and worse overall survivals after platinum/taxane-based regimens (Bamias et al. 2010; Goff et al. 1996). It is not known whether histological parameters

per se affect tumor behavior, including the responses to platinum/taxane therapy, but as manifestations of intrinsically different molecular diseases they could be considered as semaphores in determining appropriate therapeutic courses or in highlighting the need for development of more effective regimens. For example, the resistance of the ovarian clear cell subtype to conventional platinum-based chemotherapy has encouraged further investigation into the unique aspects of its molecular pathogenesis (Tan et al. 2013). When using histological subtype or grade to determine the therapeutic course or progress of therapy, it is important to consider that chemotherapy itself can significantly alter the histological of ovarian tumors (McCluggage et al. 2002).

A recent classification of ovarian cancers into two types considers grade and histology, but emphasizes molecular lineage as the prime determinant of tumor behavior (Shih Ie and Kurman 2004). Type I ovarian cancers are of lower grade, often confined to the ovary, and are thought to arise from a precursor lesion (e.g., adenofibromas or borderline tumors) by stepwise progression through a relatively well-defined series of mutations (Lim and Oliva 2013). Type I tumors in comprise low-grade serous, low-grade endometrioid, clear cell, and mucinous carcinomas, and Brenner tumors. Type II tumors are of higher grade, and are thought to arise from an initial event causing genomic instability, followed by clonal expansion and additional mutational hits (Landen et al. 2008). The factors initiating genomic instability may be associated with changes in the status of TP53, which is predominantly abnormal in Type II ovarian tumors, whereas in Type I tumors it is normal (Ayhan et al. 2009; Roh et al. 2010). In addition, genomic instability may be promoted in Type II tumors by defects in BRCA1 or BRCA2. In a study of high grade serous ovarian cancers with TP53 abnormalities, defects in BRCA caused by either germline mutations, somatic mutations, or methylation was observed in 50% of cases (McAlpine et al. 2012). Further, defects in BRCA were not observed in a group of non-high-grade serous cases. Although TP53 abnormalities could be considered a signature for high grade serous ovarian tumors, only a subset of these tumors display BRCA abnormalities, and the functional relationship between BRCA and TP53 abnormalities in ovarian cancers is not established. It is reasonable to suggest that loss of BRCA DNA repair pathways through genetic or epigenetic mechanisms may promote the generation of TP53 defects in the precursor cell population of high grade ovarian carcinomas. As genomic destabilization appears to be an essential early event for development of Type II ovarian carcinomas, defects in TP53 may be considered driver mutations in these malignancies.

Overall, Type I ovarian tumors are more indolent than Type II, with those presenting clear cell histology being the most aggressive. A two tier grading system of serous ovarian cancers (Malpica et al. 2004) revealed that survival of patients with low grade tumors (Type I) was significantly higher than those with high grade tumors (Type II). In addition, patient with Type I tumors have a better prognosis following surgery (Braicu et al. 2011). On the other hand, clinical and in vitro evidence has revealed that primary and recurrent Type I ovarian cancers are less responsive to platinum/taxane-based chemotherapy than Type II (Santillan et al. 2007). As indicated above, advanced clear cell or mucinous ovarian cancers respond poorly to cisplatin/taxane treatment (Bamias et al. 2010; Goff et al. 1996). Because of the more indolent nature of many Type I ovarian cancers, however, some studies have shown median overall survival time of patients treated with platinum/taxane is higher for Type I than Type II ovarian cancers (Bamias et al. 2012), an observation significantly affected by the higher baseline survival of patient with Type I cancers. The prevalence of TP53 defects in Type II ovarian cancers, and their absence from Type I cancers, suggests that in ovarian cancer cells TP53 function may be enigmatic, and protect cancer cells against the cytotoxic effects of platinum chemotherapy. Depending on the cellular setting, TP53 promotes apoptosis, senescence or cell cycle arrest. Functional TP53 cooperates with chemotherapy when the therapeutic agent activates its functions in apoptosis or senescence. Chemotherapeutic regimens that

activate the cell cycle arrest function of TP53, however, may be more effective in TP53-defective tumors, as cell cycle arrest by TP53 can confer resistance (Bunz et al. 1999; Johnson and Fan 2002). While TP53 activation may contribute to the cytotoxic response of normal cells such as kidney epithelium to platinum (Wei et al. 2007), the cytotoxic response of Type II ovarian cancers to platinum reagents must involve TP53-independent pathways. In fact, the wild-type TP53 characteristic of Type I ovarian cancers may induce cell cycle arrest and provide a protective effect. Consistent with this notion, some studies have shown TP53 defects to be positively associated with survival in the short term (Hall et al. 2004).

Low grade Type I ovarian cancers are associated with a prevalence of defined genetic changes in certain signaling pathways, including mutations in *KRAS*, *BRAF* and *ERBB2* and activation of MAPK signaling in low grade ovarian serous carcinoma (Anglesio et al. 2008; Sundov et al. 2013); mutations of *PIK3CA*, inactivation of *PTEN*, and activation of phosphatidylinositol 3-kinase/AKT pathways in ovarian clear cell carcinoma (Tan et al. 2013); and *CTNNB1* (β-catenin), *PTEN* mutations, and activation of Wnt/β-catenin signaling pathways in low grade ovarian endometrioid cancers (Geyer et al. 2009). As Type I ovarian cancers respond poorly to platinum chemotherapy, arise from identifiable progenitors, alter specific signaling pathways during progression, and display genetic stability, efforts to profile genetic or expression markers that would predict outcomes of individual Type I ovarian cancers after platinum chemotherapy have been limited. In contrast, Type II ovarian cancers arise from cryptic precursors, activate a wider range of signaling pathways and display genetic instability. Type II ovarian cancers initially respond well to platinum/taxane adjuvant therapies, but eventually recur after a range of disease-free intervals. Identification of gene expression or immunochemical markers with predictive value in determining disease free interval or survival in independent or multiplex analyses would provide a means of tailoring treatment regimens or of identifying patients that would require novel therapies. Studies of platinum drug resistance often do not distinguish between Type I and Type II ovarian cancers, and therefore end up characterizing molecular differences between the cancer types rather than specific differences that may contribute to variations in the responses of individual tumors.

As TP53 mutations are present in most type II ovarian carcinomas, and the few that have nominal TP53 activity may have copy number gain in MDM2 or MDM4, TP53 status is not a useful predictor of drug resistance or clinical outcome in these cancers (Ahmed et al. 2010). On the other hand, defective BRCA 1 or 2 is observed in only 10% of all ovarian cancer cases, and defects in BRCA 2 are associated with better survival and therapeutic response than those bearing defective BRCA 1 or wild-type BRCA genes (Liu et al. 2012; Yang et al. 2011). Further prognostic breakdown of high grade cancers using gene expression profiling approaches, however, has proved challenging. The expression pattern of a panel of 11 genes has been shown to have predictive value in determining survival of patients with high grade serous treated with carboplatin and paclitaxel (Gillet et al. 2012). A connection between clinical endpoints and genetic alterations in high grade serous type ovarian carcinomas has noted eight regions of amplification or deletion on five chromosomes that clustered into subgroups, suggesting that high grade serous cancer may be segregated into clinically distinct subgroups (Engler et al. 2012).

Platinum Resistance and Ovarian Cancer Recurrence

Mechanisms of Platinum Drug Resistance

Diverse molecular mechanisms have been proposed to contribute to the acquisition of resistance to organoplatinum compounds and other therapeutic agents, including modulation of drug uptake and efflux, enhanced mechanisms of detoxification, inhibition of apoptosis, and recovery or enhancement of DNA repair mechanisms.

Genes or gene products observed to affect cisplatin sensitivity of cancer cells include: metallothionein, thought to be an intracellular metal sink; CCND1, a G_1 cyclin (Noel et al. 2010); ERCC1, an enzyme involved in DNA excision repair (Damia et al. 1996); glutathione S-transferase (GST), thought to modulate signal transduction kinase cascades in response to stress (Townsend et al. 2005); and interleukin 6, a cytokine (Wang et al. 2010). Modulators of apoptosis, including TP53, X-linked inhibitor of apoptosis protein, and Akt have been found in cultured ovarian cancer cells to be interdependent determinants in the response to cisplatin (Abedini et al. 2009), and alterations in the TP53 gene have been associated with variable responses to cisplatin of cultured ovarian cancer cell lines (Perego et al. 1996). Since the majority of type II ovarian cancers contain defective TP53, the relevance of cell culture studies of TP53 in differential responses of high grade ovarian carcinoma cells to cisplatin remains unclear. Finally, in addition to cell autonomous mechanisms, the tumor microenvironment is thought to play an important role in determining chemoresistance of ovarian cancers (Chien et al. 2013).

Defects in FANC-BRCA pathways are associated with genomic instability and enhanced sensitivity to platinum drugs in a subset of ovarian carcinomas. During oncogenic progression, methylation of FANCF or BRCA1 occurs in a subset of ovarian carcinomas (perhaps as high as 20%), resulting in genomic instability, and thereby promoting other neoplastic changes (Taniguchi et al. 2003). Subsequent reversion of these genes to demethylated forms restores drug-resistance to this subset of cancers. Familial forms of ovarian carcinoma represent 10–15% of cases, are associated with inherited defects in BRCA1/2 that compromise DNA repair functions, and are hypersensitive to platinum reagents. While the prognosis of cisplatin treatment is better for patients developing BRCA1/2 defective ovarian carcinomas, these cases relapse into drug-resistant forms, frequently driven by secondary (reversion) mutations in BRCA1/2 (Sakai et al. 2008; Swisher et al. 2008). Somatic mutation of BRCA1/2 in sporadic ovarian carcinomas is rare, and while changes in BRCA1/2 expression also should be considered, other distinct mechanisms undoubtedly contribute to drug resistance.

Epigenetic Contributions to Platinum Drug Resistance

The epigenetic machinery of the cell includes DNA methylation, histone modifications, non-coding RNAs, and chromatin remodeling and organization, the latter being affected by the first three as well as by the architecture of the nucleus. Epigenetic regulatory mechanisms may work in concert with somatic mutations to drive tumor progression and promote tumor cell survival. An association has been noted between CpG island methylation of the MLH1 mismatch repair gene and relapsed invasive ovarian cancers, and this can be reversed in xenographic models by demethylating agents (Zeller et al. 2012). There is clinical evidence that the hypomethylating agent azacitidine can partially reverse platinum resistance in patients with ovarian cancer (Fu et al. 2011). Low dose decitabine administered before platinum to resistant ovarian cancers has been shown to alter methylation of MLH1, RASSF1A, HOXA10 and HOXA11 and positively correlate with progression free interval (Matei et al. 2012). Intriguingly, recent studies have suggested that HDAC4 is enriched post-treatment in cisplatin resistant cells of high grade serous ovarian cancers, and HDAC4 promotes cisplatin resistance through deacetylation of STAT1 (Stronach et al. 2011). Contingent on gene and tissue type, metazoan genes localized adjacent to the nuclear envelope generally tend to be suppressed, whereas genes localized centrally in the nucleoplasm tend to be transcriptionally active (Malik et al. 2010). Chromatin organization appears to be influenced by its proximity to the NPC, as channels of euchromatin interrupt the lamina and extend from the NPC into the nucleus. Alterations in nuclear pore structure, possibly by affecting the architecture of underlying chromatin, may play an important role in determining sensitivity or resistance of ovarian

cancer cells to platinum-based chemotherapy (Kinoshita et al. 2012).

Regrowth of tumors from remnant tumor cells present an important challenge to the success of cancer chemotherapy. Adding to the complexity of this challenge, the expression of drug resistance by remnant tumor cells does not appear to depend on genetic modifications of tumor cells, but rather may be conferred by ephemeral epigenetic changes that can disappear upon regrowth of the tumor. Thus, growth of remnant cells does not necessarily result in the generation of chemoresistant tumors. A study of retreatment of ovarian cancer patients with platinum drugs after remission and relapse with the same regimen of chemotherapy revealed surprisingly high probabilities of success (Seltzer et al. 1985). A review of retreatment of relapsed tumors of different types revealed that transient resistance to chemotherapy is common (Cara and Tannock 2001), and statistically discounted somatic mutation as the prevailing mechanism, suggesting instead epigenetic modalities. Recent studies of tumor cells in culture have shown that populations of drug-tolerant cells persist after treatment with different chemotherapeutic agents, and that these cells are mostly quiescent and express surface markers in common with cancer stem cells (Sharma et al. 2010). When these cells remain in culture in the presence of drug, a fraction give rise to colonies of cells with relatively stable tolerance, and that cell population remains tolerant in the presence of drug and for many generations after drug removal. The frequency with which tolerant and stably tolerant cells appeared in these experiments also suggest mechanisms that do not involve genetic mutations, and further studies indicated that chemical agents that inhibit histone demethylation also block acquisition of drug tolerance, suggesting an epigenetic mechanism (Sharma et al. 2010).

Profiling of microRNA (miR) expression in ovarian cancer patients treated with platinum-taxane therapy identified a signature of miRs 484, 682, and 214 that predict sensitivity, and that miR484 operates by modulating tumor vasculature through modulation of VEGFB and VEGFR2 pathways (Vecchione et al. 2013). Although no difference was observed among serous tumors, miR-141 (which targets KEAP1, a gene involved in oxidative stress) levels were higher in non-serous ovarian tumors that did not respond well to therapy (van Jaarsveld et al. 2012). miR130a is upregulated in cisplatin-resistant cultured cell lines, and may be indirectly associated with MDR1 and P-glycoprotein conferred resistance (Yang et al. 2012) Two microRNAs, miR-152 and miR-185, increase the sensitivity of cultured ovarian carcinoma cell lines to cisplatin in vitro and in vivo, and have been found to target DNA methyltransferase 1 directly (Xiang et al. 2014).

Cancer Stem Cells, Cellular Dormancy and Recurrence of Ovarian Cancer

The cancer stem cell (CSC) hypothesis arose from the concept that tumor cells of a single genotypic origin can display different cancer phenotypes. Historically, transplantation of tumor cells between animals or between cultures plates and animals was observed to require a larger number of cells than would be predicted from the clonal nature of the tumor, and further studies revealed that subpopulations of cells could be selected or sorted from the total tumor cell population that were more efficient at initiating new tumors. These cells comprised a small fraction of the total tumor cells, and similar to embryonic stem cells, have the capacity for self-perpetuation as well as for giving rise to other types of cancer cells. Cell surface marker, transcriptomic, and signaling systems analyses revealed similarities as well as differences between CSCs from different tumor types, as well as similarities between CSCs and embryonic stem cells or induced pluripotent stem cells (Schoenhals et al. 2009). To account for the self-renewal of CSCs and the accumulation of the tumor mass of non-CSCs, CSCs are thought to undergo asymmetric mitoses, yielding both CSCs and the "differentiating" cells that comprise the majority of the tumor load (Fig. 7.2). The latter may proliferate more rapidly than CSCs, but eventually become terminal. Positive correlations between the prevalence of cells

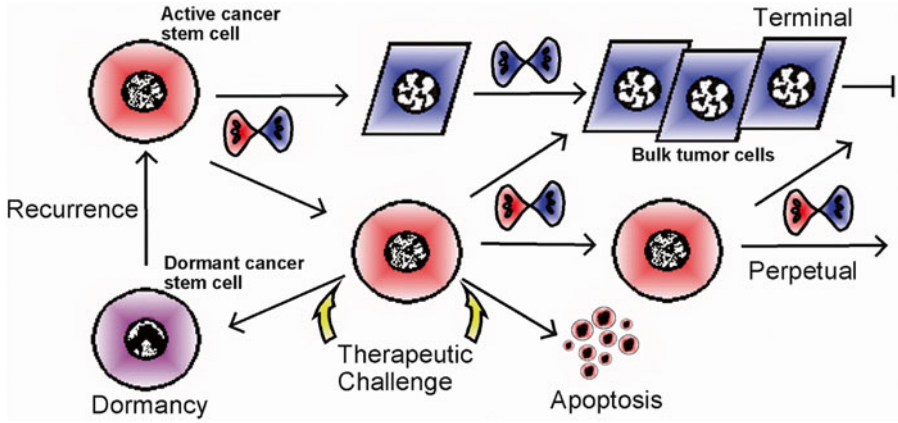

Fig. 7.2 Defining the relationships between cellular dormancy, tumor recurrence, and cancer stem cells. *Red*: actively growing cancer stems cells; *blue*: bulk tumor cells; *purple*: dormant cancer stem cells. Asymmetric mitoses are indicated as *red/blue*

bearing CSC markers in ovarian cancers and recurrence have been reported (Steffensen et al. 2012). Cancer stem cells for epithelial ovarian cancers may be the source of progenitor cells for metastases. Metastasis of CSCs is promoted by epithelia to mesenchymal transition, a process evidenced by their ability to form spheroids, and subsequent mesenchymal to epithelial transitions, and both processes appear to be mediated by changes in the intracellular stability of TWIST1 (Yin et al. 2013).

Cancer stem cells have been identified in epithelial ovarian malignancies by several methods. In FACS analyses, a side population of ovarian tumor cells that excludes Hoechst dyes has been shown to display enhanced tumor-initiating properties in xenografts (Szotek et al. 2006). Cell populations with tumor-initiating properties have been isolated from ovarian cancers using positive selection with surface markers such as CD44. Marker analyses have revealed that tumor initiating cells phenotypically resemble CSCs from other tissues, expressing some of all of the following: CD44, CD24, Epcam, CD133, ALDH1, MyD88, and CD117 (Kryczek et al. 2012; Meirelles et al. 2012; Steffensen et al. 2012), as well as of pluripotency markers such as β-catenin, Oct-4, and SSEA-4 (Alvero et al. 2009b). Loss of expression of other markers, such as Ecadherin (Meirelles et al. 2012), also are associated with the CSC phenotype in ovarian tumors. While genetic defects in β-catenin have been linked with low grade endometrioid cancers, they are rare in serous and other ovarian carcinomas (Geyer et al. 2009). Nonetheless, activation of Wnt/β-catenin pathways in epithelial ovarian cancers are associated with the CSC phenotype (Arend et al. 2013), and a correlation between activation of signaling pathways involved in epithelial to mesenschymal transitions and those activated in CSCs is apparent (Talbot et al. 2012). Recent studies have found that blocking the Notch signaling pathway, which is involved in epithelial to mesenchymal transitions, improves the response of ovarian cancer to platinum therapy (McAuliffe et al. 2012).

The CSCs phenotype is relatively chemoresistant in compared to the bulk tumor cell population, and epigenetic mechanisms appear to function in both chemoresistance as well as in perpetuation of the stem cell phenotype (Alvero et al. 2009a; Crea et al. 2009; Steg et al. 2012). Primary/recurrent pairs of high grade ovarian adenocarcinomas were analyzed for expression of cancer stem cell markers (ALDH1A1, CD44, CD133). Immediately after primary therapy (combination: cisplatin or carboplatin and taxane (either paclitaxel or docetaxel), the percentage of remnant tumor cells expressing one or more of these markers was enriched, with

CD133 displaying the greatest degree of enrichment. Some stem cell transduction pathways also were observed to be enriched the recurrent tumors (Steg et al. 2012). It has been suggested that the enhanced expression of selective transporter ABCG2 and MDR1, as well as expression of toll-like receptor signaling may function in chemoresistance of ovarian CSCs (Fong and Kakar 2010).

Exit of chemoresistant tumor cells from the cell cycle is sometimes referred to as cellular dormancy in order to distinguish it from other events that can result in blocked or retarded tumor growth, such as insufficient vasculature or attack by the immune system (Aguirre-Ghiso 2007). Tumor cellular dormancy can follow therapeutic intervention, but is also observed in primary tumors, as their growth can lag or stall before they become clinically significant (Harach et al. 1985). Protracted tumor dormancy often occurs after treatment; and tumor cells that resist therapy can persist in an occult or asymptomatic state for years before causing a recurrence of disease (Udagawa 2008). How cancer cells produce a chemoresistant dormant population, such as through asymmetric tumor growth or through induction by exposure to therapeutic agents, is not understood. Equally elusive are the mechanisms that induce dormant (quiescent) cancer cells to awaken and proliferate, resulting in recurrence.

It is important to define tumor cellular dormancy in the context of the CSC hypothesis (Fig. 7.2). The activity of CSCs would likely be impacted by factors that restrict tumor growth, such as vascular insufficiency or immunological intolerance, but in these cases dormancy results from the equilibrium reached between tumor growth and attrition due to lack of nutrients or negative selection by the immune system. In contrast, tumor cellular dormancy after therapeutic intervention is associated with protracted cell cycle arrest, and is a state associated with tumor cells that have survived therapeutic challenge. By definition, dormant tumor cells are dormant CSCs. For tumor cells in this state to be properly referred to as dormant and to be distinguished from terminal senescent cells, they must have the capacity to reenter the cell cycle and clonally support regrowth and recurrence of the tumor. Thus, merely spotting quiescent tumor cells in tissues after chemotherapy and remission does not necessarily identify them as dormant, as the capacity of these cells for regrowth would remain uncertain.

The CSC hypothesis predicts that bulk and other types of tumor cells arise from asymmetric division of CSCs into CSC and bulk tumor cells, followed by rapid but limited growth of the resultant bulk tumor cells (Fig. 7.2). Some evidence has suggested that prevalence of cells bearing CSC markers in an epithelial ovarian cancer correlates with positively with recurrence (Steffensen et al. 2012). Little is known, however, of the relative frequency of symmetric and asymmetric divisions of CSCs, whether these frequencies vary at different periods of tumor growth, or whether the growth parameters of CSCs are similar to the those of dormant tumor cells in the early stages of tumor recurrence. Increases in the serum CA125 antigen have been used to detect early recurrence of ovarian cancers, but no survival benefit was found for early versus delayed treatment of recurrent tumors (Rustin et al. 2010). Since the tumors in this study were recurrent rather than newly diagnosed, it is unlikely that the lack of a survival benefit is due to overdiagnosis and/or overtreatment (Klotz 2012). Rather, this surprising outcome suggests an absence of knowledge of the kinetics of tumor growth, in particular the growth of tumors from dormant cells. For example, does malignant tumor growth from single dormant cells produce consistent fractions of bulk tumor cells and CSCs, or does growth proceed through phases in which growth of one population outpaces the growth of the other? Enhanced accumulation of the CSC fraction during early tumor growth could explain the lack of a survival benefit for early treatment of recurring tumors.

The lack of controlled and defined model tumor cell systems has precluded directly studying the cellular kinetics and dynamics of tumor growth from dormant cells. After drug treatment of a tumor in a rodent model, investigators may need to wait 300 days or longer to observe recurrence, and as such are limited by the practical

duration of the experiment as well as the natural lifespan of the animal. As indicated above, the high probability of recurrent tumors responding to retreatment with the original platinum-based chemotherapeutics suggests that transitions between drug-resistant, dormant, and malignant states are driven by epigenetic events, and this make sense as dormant cells are unlikely to undergo rounds of somatic mutation and selection as would be required these transition were driven by genetic events. Studies of some ovarian cancer cell lines have suggested that alterations to nuclear pore architecture, and underlying changes in chromatin architecture, directly affect sensitivity to platinum drugs, and can be employed to drive transitions between cellular dormancy and malignancy in an experimental system (Kinoshita et al. 2012). These results are consistent with epigenetic modalities regulating platinum drug resistance and cellular dormancy, and suggest that variation in expression of nucleoporins can produce phenotypic diversity within the tumor cell population. Although dormant tumor cells must function as CSCs in order to fuel recurrence of a tumor, it is not necessarily clear that they arise or are selected from the pool of CSCs. Little is known of the potential overlap in phenotypic markers between dormant tumor cells and CSCs, nor whether an abundance of cells expressing CSC markers in a particular tumor would predict a greater likelihood of generating dormant tumor cells in response to therapy. It has been reported that ovarian cancers with a greater percentage of cells expressing certain CSC markers (e.g., CD133; (Zhang et al. 2012)) have a poorer prognosis; however, it not known whether this is because of their intrinsic chemoresistance or because, in response to therapy, they yield a greater fraction of dormant cells with tumor-initiating properties.

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