Chapter 5

Genomic Copy Number Alterations in Serous Ovarian Cancer

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Additional information is available at the end of the chapter

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Abstract

Precision medicine in cancer is the idea that the recognition and targeting of key genetic drivers of a patient's tumor can permit more effective and less toxic outcomes. Point mutations that alter protein function have been primary targets. Yet in ovarian cancer, unique genetic mutations have been identified only in adult granulosa cell tumors, with a number of other point mutations present in mucinous, clear cell and endometrioid carcinoma subtypes. By contrast, the serous subtype of ovarian cancer shows many fewer point mutations but cascading defects in DNA damage repair that leads to a network of gains and losses of entire genes called somatic copy number alterations. The shuffling and selection of the thousands of genes in serous ovarian cancer has made it a complex disease to understand, but patterns are beginning to emerge based on our understanding of key cellular protein networks that may provide a better basis for future implementation of precision medicine for this most prevalent subtype of disease.

Keywords: SCNA, aneuploidy, autophagy, beclin-1, p53

1. Introduction

When a patient asks an oncologist what tumor cells are, the frequent explanation is that the "Cancer cells are normal cells that accumulate genetic mutations, which causes them to grow out of control." Yet the idea of what a mutation is, and what it can do, varies. It has essentially become dogma that mutations be grouped into two broad categories. One class has been described as either *drivers*, which are key genetic changes that are known to potentiate tumor development. If a gene is not a driver, then it is typically considered a *passenger*,—a bystander mutation occurring due to the tumor-associated genomic instability. Passenger mutations are generally considered to be 'noise' in the system which do not influence tumor progression [1].

This categorization has now had clinical impact. Genes that are known as *drivers* are prioritized for diagnostic testing, and have become a focus for "molecular tumor boards" that review patient data in hospitals across the United States. These boards focus foremost on reviewing a molecular profiling of the tumor, rather than on histopathological features. Thus, tumors with similar genetic features may call for similar therapy regardless of whether they originate in the colon, breast or lung. The division of mutation into drivers and passengers fosters an environment where new mutations may be missed, because we are focused on the pre-established clinical screening protocols, because we both profile and act upon well characterized genetic problems. Even when they are reported, their impact may not be appreciated if they have not had a role as a driver assigned to them in prior peer-reviewed study.

The *driver* assignment comes from a breadth of work that focuses on a type of mutation called a somatic single-nucleotide variant (SNV). Driver SNVs are noted for their critical roles in tumor formation, frequently occur at precise locations within *oncogenes*, and can now be rapidly identified. Notable examples include K-Ras, where mutation of the glycine residue at position 12 (G12) inhibits GTPase activity, leaving the protein in an active, GTP-bound effector state. A second example is phosphoinositide 3'kinase, where mutation of the histidine residue at 1047 (H1047) similarly alters the ability of the protein to regulate activity. The gold standard for such driver mutations is their capacity to facilitate neoplastic disease in murine genetic models, most frequently by providing a dysregulated positive stimulus that drives mitosis and cell survival. Transcription factor mutations, such as the FOXL2 C243W mutation found in all adult type granulosa cell tumors, provide a good example of a key genetic driver.

A second class of drivers involve SNV-mediated inactivation of *tumor suppressor* genes, which act to ameliorate the effects of oncogenes, shunt tumors towards programmed cell death, and maintain the fidelity of DNA replication and repair. Tumor formation requires both oncogenic activation and the disruption of tumor suppressors. Mutation in TS genes do not require the same precision as those in oncogenes; SNV's occur across a swath of locations, any of which may be sufficient to disrupt tumor suppressor function. This chapter will focus on serous

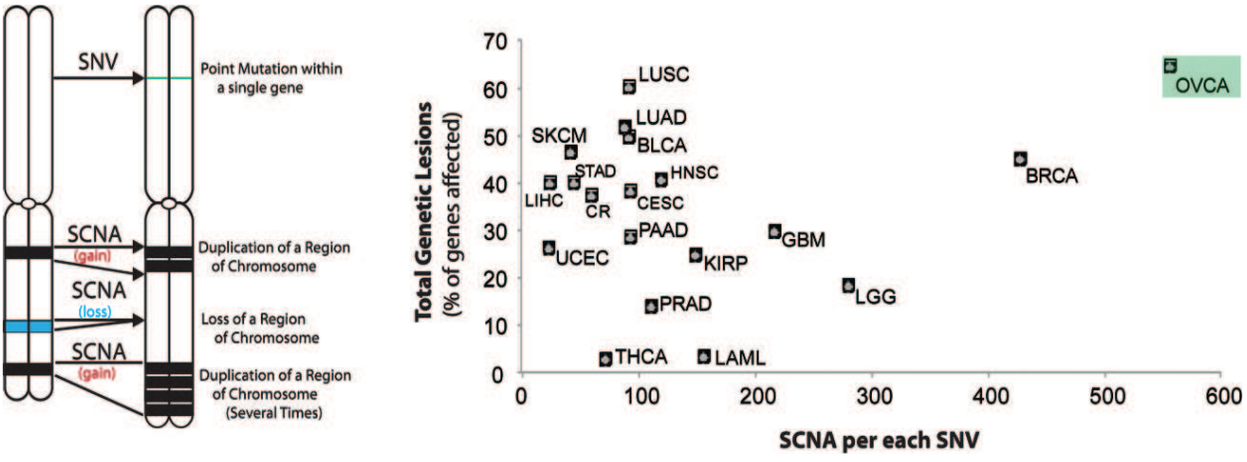

Figure 1. (Left) Possible changes in a single chromosome's architecture. One chromosome is shown; each copy of a chromosome can have different SCNA or SNV. (Right) A plot of the major cancers in the TCGA database, showing total genetic lesions (as percentage of genes) vs. the number of SCNA for each SNV. In each case SCNA are more abundant, with serous ovarian cancer (SOC, denoted as OVCA in the green box) bearing the greatest number.

ovarian cancer (SOC), a lethal tumor whose '*drivers*' are only beginning to be understood. The tumor suppressor gene *TP53* is mutated in more than 85% of serous ovarian cancer (SOC) cases [2], and disruption of DNA repair proteins is commonly identified. Yet most patients bear no SNV that results in oncogene activation.

However, SOC has a further characteristic related to its poor capacity to repair DNA. SOC has the highest ratio of somatic copy number alterations (SCNAs) to SNVs for any major cancer. SCNAs are a broad group of genetic changes that encompass a myriad of short insertions, short deletions, translocations and inversions (**Figure 1**, left panel). SCNAs contribute to the mutational landscape of cancer, expanding the scope of changes beyond the more 'simple' SNVs. The impact of this on SOC malignancy will be the focus of this chapter.

2. SCNA overview and incidence

A gene normally occurs in the human nucleus twice. This normal 2N "dosage" of copy number, which originates from zygote formation, consists of one paternal gene and one maternal gene. SCNAs, which alter this occur in two types: amplifications and deletions. An amplification occurs when a chromosomal region containing a gene is copied. That gene will no longer have the normal 2N copy number, but, depending upon the number of times it is copied, could be 3N, 4N, or in cases of massive amplification, up to 200 N and more. Contrasting with this expansive range, SCNAs that result from deletions most frequently reduce the copy number to 1N. Total gene loss (0N) can occur in rare cases, and is associated with a very small fraction of the overall number of deletions. Nonetheless, these rarer SCNA-derived genotypes will obviously impact function most, since the lack of any gene copy means that the encoded protein cannot be produced. SCNAs are the most common lesions in cancer, occurring much more commonly than SNVs (**Figure 1**, right panel).

SCNAs occur via a variety of mechanisms in cancer [3]. Entire chromosomes may be gained/lost during cell division, generating 3N or 1N copy number status for all genes on the chromosome. This occurs due to failed cell-division checkpoints resulting in chromosome missegregation. In contrast to such gains at the total chromosome level, tiny "focal" SCNAs may alter a single gene (or even part of a gene). The most common example of this is *CDKN2A*, a checkpoint protein which is fully deleted (0N) in 3% of SOC tumors. These focal deletions typically occur during repair of double-stranded DNA (dsDNA) breaks. During the attempted repair, short regions of homology can result in accidental deletion of DNA in between [4]. Focal amplifications occur through unknown mechanisms [5] and can form "double minute" chromosomes containing hundreds of copies of a gene, such as *ERBB2* or *EGFR* [6]. Finally, between the focal alterations and the whole chromosome losses, SCNAs can also encompass large regions of DNA through similar defects in dsDNA break repair. These intermediate sized SCNAs can contain many genes. However, they rarely contribute to a 0N copy numbers (loss on both chromosomes) since the regions affected frequently contain essential genes [7].

Within the Cancer Genome Atlas (TCGA) data sets, the presence of 3N and 1N gene copies dominate the SCNA genomic landscape. This is true across all tumors, including those tumors where SCNAs are highly prevalent, such as SOC [8]. SCNAs are prevalent in SOC. In

fact, only about one third of all genes in primary tumors have a normal 2N gene dosage. Roughly a quarter of the total genes in the tumors show an extra gene copy (to 3N) and just over a third lose a gene copy (to 1N). By contrast, only 0.7% loses both gene copies (0N), while 4.2% are amplified to 4N or greater. In practice, the focus on understanding tumor biology has been only on these last two cases (total deletion and gross amplification, respectively). This has a reasonable basis; the effects of total loss or gross amplification are easiest to study.

The common gene changes (i.e., 1N and 3N) have not been the subject of focused study. Many scientists assume that the deletion, or addition, of a single gene copy has limited effect. Recessive genetic alleles are not uncommon in nature, supporting the idea that the loss of a single gene copy can be compensated for. However, the loss of a single gene may not reflect the situation in ovarian cancer, where massive genetic alteration occurs, and compensation may not be possible if the same cellular pathway is repeatedly targeted by SCNAs (**Figure 2**).

More than 80% of genes affected by SCNAs show concordant alteration of mRNA levels [9, 10]. For ~70% of genes, this correlates with steady-state protein levels [11]. Thus, SCNAs offer a predictable, but not absolute, indication of protein expression. This is relevant to ovarian cancer, as SCNAs modify on average 67% of the SOC genome, whereas SNVs modify only

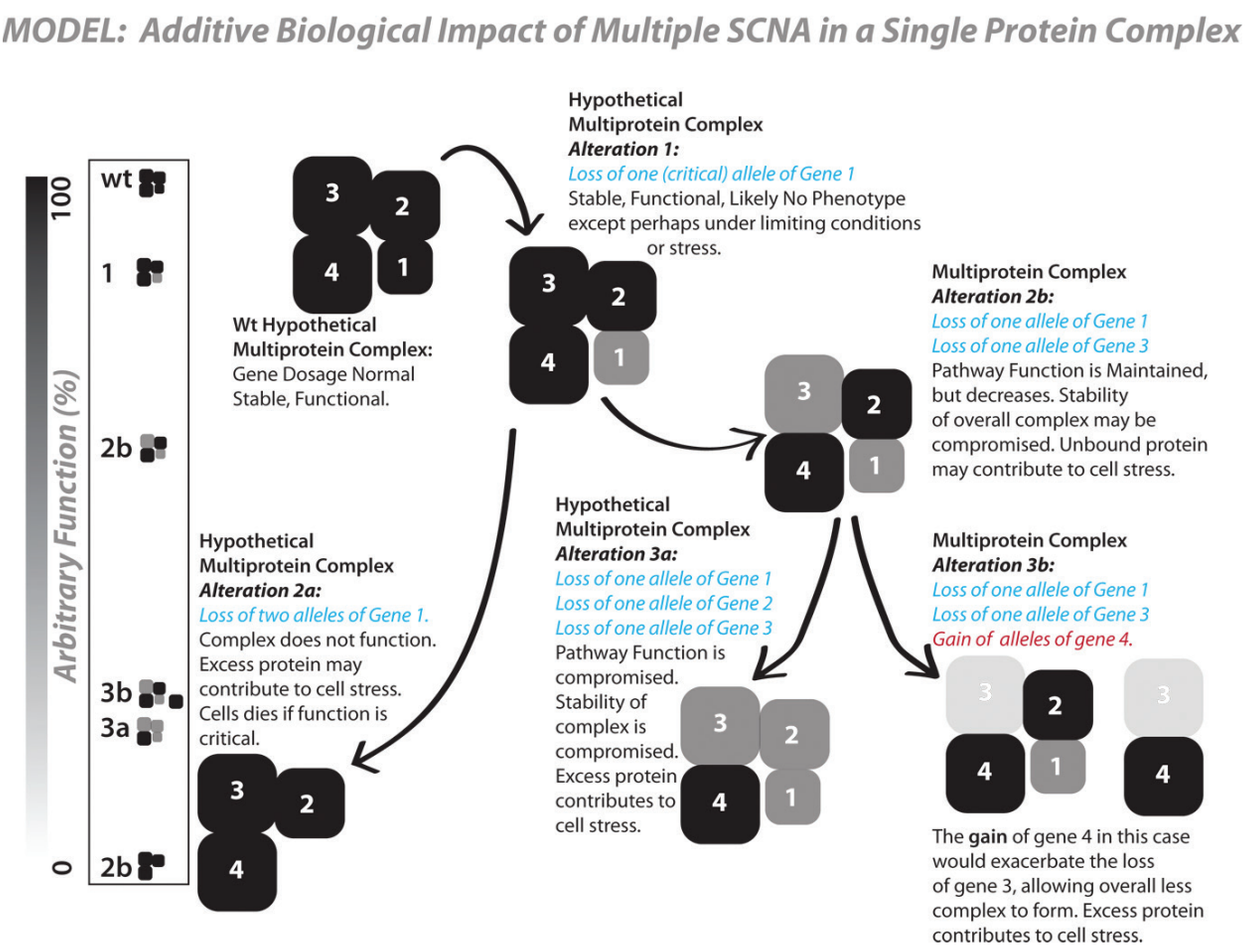

Figure 2. Model showing how SCNA changes resulting in differences in protein expression might impact overall function within a single multiprotein complex. The relative function of the complex as a % is shown at left. The right side shows how a sequence of SCNA changes within a pathway could cumulatively impact its function.

0.12% of the average SOC genome [12]. Less than 10% of SOC patients are mutated in a targetable driver gene [12, 13].

3. Ovarian cancer and copy number alterations

It seems self-evident that an understanding of "driver SCNAs" is absolutely essential to our capacity to target the biology of the disease. Genetic disorders such as Down's syndrome (trisomy 21) and Cri du Chat (5p monosomy) and DiGeorge Syndrome (loss of only 30–40 alleles on 22q11) clearly indicate the penetrative biology of multiple SCNA. More importantly, such lesions affect only ~2% of the genome, while SCNA in SOC affect 67% of genes. Other subtypes of ovarian cancer vary widely in their SCNA burden, but are typically much lower, and are associated with SNVs.

As most SCNA are "monoallelic" changes resulting in a 1N or 3N genotype, is there any reason to expect a phenotype, given our understanding of recessive alleles? Recurrent patterns in serous ovarian cancer suggest that frequently affected regions may be selected for as the tumor evolves. In high grade SOC, the most prevalent SNVs could have been predicted from literature preceding the genomics era. For decades, the mutation of *TP53,* the "guardian of the genome" has been appreciated due to its master control of multiple DNA repair pathways, cell cycle control, and metabolism. Interestingly, there is selection for SCNA deletion of the chromosome with the wild-type copy of p53, suggesting further suppression or misdirection of p53 furthers SOC development [14]. Inheritance studies have associated the *BRCA1/2* mutation with an increased risk of ovarian cancer, and not surprisingly these mutants contain opposite-chromosome deletions just like p53. *BRCA* genes are necessary to maintain the genome. Like p53, they play a coordinating role in facilitating homology-directed repair of DNA. However, single nucleotide variant mutation is not the most common mechanism of BRCA gene disruption. Only ~6% of patients display non-germline SNVs, while copy number deletions (to 1N) occur in more than 70% of tumors. PTEN, a tumor suppressor commonly mutated in many tumor types but not ovarian, was found as early as 2001 to have reduced expression due to shallow deletions across ~40% of samples [15].

Aside from very infrequent gene losses paired with mutations, there are also a few SCNAs which drive cancer through amplification of oncogenes. The stem-cell transcription factor *MYC* is the most amplified gene in the TCGA cohort (42% with at least a 4N copy number, and an additional 37% with 3N). Myc has been appreciated as a common SOC driver oncogene since 1990 [16]. Homozygous deletions in Rb were discovered around the same time [17, 18], and occur in 9% of tumors. *KRAS* amplifications and gene overexpression were discovered around the same time, but in a smaller minority (13%) of patients [19]. Her2, encoded by *ERBB2*, can be overexpressed but this appears to be a case unrelated to SCNA amplification, which occurs in only 3% of cases [20, 21]. Drug resistance can occur following increases in drug efflux genes, and one of the first identified was *MDR1* (*ABCB1*) [22]. Again, this is only found in a small minority of patients (4%). Comparative genomic hybridization in 2006 identified significantly amplified CCNE1 (cyclin E1) and MDM2 (a negative regulator of p53 from its E3 ubiquitin ligase activity) [23, 24]. The year these studies were published provides

a historical context to our knowledge. Were we missing a key driver for SOC? Despite the hundreds of genomes sequenced, few additional single-gene drivers were discovered in the recent "brute-force" landmark studies on SOC from either the TCGA [12] or the AOC [13].

There are plausible reasons for this. It may be that every SOC tumor is truly unique from a mutational standpoint: that those SNVs found in only one tumor nonetheless are driver genes, collaborating in ways that we understand poorly [25]. It is thus possible that drivers have already been sequenced and annotated by SCNA studies, but due to high "background" or "passenger" SCNAs it remains unclear which SCNAs are critical to the tumor's biology [8]. The implications of this are enormous, and would necessitate an unparalleled level of personalized therapies targeting such extremely rare mutations. A second reason that SNVs have not yielded common drivers may be that further sequencing of whole genomes and epigenomes will reveal additional drivers prevalent across patients which have remained undetected by exome sequencing.

The problem investigators consistently encounter is the ubiquitous heterogeneity in SOC. Heterogeneity exists at all levels of genetics, manifesting as *between-patient* heterogeneity, *between-tumor* (intra-patient) heterogeneity [14], heterogeneity in SNVs within a single tumor [12], heterogeneity in SCNAs [26], and heterogeneity in mRNA expression (correlating with protein expression) [11] or flux in SCNA status [10]. While such problems are not unique to SOC, they are magnified compared to many other tumor types because of the gross incidence of SCNA. Genetic and phenotypic heterogeneity remains the hardest issue to tackle [27, 28], and our own opinion mirrors that of several other groups working in this area: the analysis of affected pathways will offer new approaches to find hidden patterns of tumor suppressors and oncogenes within these heterogeneous data [8, 9, 29].

Fewer genomic studies have been performed on other types of ovarian cancer. Some limited data are available on SCNAs for Clear cell and endometrioid subtypes, which share the amplification of PIK3CA and the MYC-containing 8q24 region with SOC [30–32]. Larger SCNAs encompassing whole chromosome arms rather than smaller changes dominate the clear cell ovarian cancer SCNA landscape [32]. With the exception of 17p loss (containing *TP53*), focal *TPM3* amplification, and focal *ERBB2* amplification, SCNAs are infrequent in mucinous ovarian cancer, suggesting this histotype is SNV or epigenetic driven [30]. Generally, clear cell and endometrioid are intermediate in SCNA quantity between SOC and mucinous subtypes of ovarian cancer.

Despite the limited data on these non-serous subtypes, there is good reason to expect much more data is coming soon. The copy-number arrays employed in the Cancer Genome Atlas studies sell for less than $100USD per sample, which bests the current, but constantly decreasing, cost of whole-genome sequencing. Eight oncology treatment and research centers are participating in project GENIE, which has just released 19,000 new tumor datasets to the public and will continue to grow [33]. As sequencing becomes a normal part of the treatment strategy for patients, the number of samples will likely outpace scientists' ability to fully analyze and comprehend the complex data. Nonetheless, gathering these data is essential to progressing our understanding of the differences between cancer subtypes, which will facilitate the matching of pharmaceuticals to genotype. For now, the largest datasets exist in SOC, and will be the focus of the remainder of discussion.

4. The interplay of p53 mutation with copy number instability

Mutation in p53 has a long research history in many cancer types, and ovarian cancer is no exception. Ovarian cancer mutations within *TP53* have been observed since 1991 [34], and have been confirmed in every genetic study since. *TP53* has often been referred to as "the" primary tumor suppressor for its central role in responding to stresses: it can halt the cell cycle, divert metabolism, induce transcription of DNA damage response genes, or if the damage cannot be repaired, induce apoptosis or senescence [35–37]. For serous ovarian cancer, it has been used as a marker for false diagnosis as some studies presume that genuine serous ovarian cancers must contain mutant p53. Similarly, since the beginning of genomic copy number studies using comparative genomic hybridization, SCNAs have been labeled as a ubiquitous event in all types of epithelial ovarian cancer [31]. Not all p53 mutant tumors are high in SCNAs [38]. Nonetheless, tumors with higher than average SCNAs are much more likely to have a facilitating mutation in p53 [26, 39, 40]. This implies a basic premise: ovarian cancer tumors utilize the mutation in *TP53* to enable the proliferation of SCNAs. SCNAs can subsequently occur in additional tumor suppressors and oncogenes, which leads to SOC as we know it.

Mechanistically this could occur via the deletion or duplication of entire chromosomes or genomes, followed by many subsequent changes enabled by the extra copies of genes, or via chromosome missegregation event, leading one or more chromosomes to acquire massive damage [41]. Either possibility can explain the high frequency of chromothripsis, a highly-disorganized form of hundreds or thousands of SCNAS, in SOC. Mutant p53 enables such mechanisms of SCNA formation by preventing the death of the cell that bears them, as missegregation directly induces p53-dependent cell-cycle arrest followed by apoptosis [42]. In one well-controlled study, 'dominant negative' p53 reduced the cell cycle delay associated with trisomy in mammalian cells, yet it was rare that gain of any single chromosome in those cells resulted in any proliferative advantage [43]. Thus, partial or gained p53 function may contribute. Many p53 mutations maintain partial function, while mutations such as R273H (the most common variant of TP53 found in SOC) provide a gain of function by directly impairing Mre11/ATM-dependent DNA damage responses [44].

It is likely that mutation in *TP53* gene is an enabling event. Lineage tracing using millions of sub-clonal passenger mutations present in SOC tumors suggest that *TP53* mutation arises very early in the proliferation of pre-tumor cells [14]. While few studies focus on normal tissue, a publication on aged skin samples found that islands of cells had developed p53 mutations and achieved local proliferation. Exceptionally few copy number alterations were revealed, with the exception of deletions in *NOTCH1*, and these lesions did not progress to malignancy [45]. In murine models, too, SCNAs follow initiating mutational stimuli [46]. The findings support the idea that p53 is likely to become mutated prior to SCNA accumulation, and acts permissively to enable SCNA accumulation.

5. BRCA1/2 mutations and homologous repair defects

Though few SNVs in 'classic' tumor genes are found in SOC relative to other cancer types, BRCA1/2 mutations are among the most frequent at ~10% [12]. BRCA genes work in

coordination with dozens of other proteins to perform genome maintenance via homologous recombination [4]. The double-stranded break repair pathway begins with PARylation of the break site by PARP1, megabases of phosphorylation of H2AX and subsequent formation of Rad51 filaments. Brca1 & Brca2 bind Rad51 to stimulate strand invasion of sister chromatids during homology directed repair. While only ~10% of SOC are mutated in BRCA1 or BRCA2, it is noteworthy that 75% of patients have lost one of two alleles of BRCA1 and 57% have lost an allele of BRCA2. Very few tumors (~1.5%) have homozygous deletions in BRCA1 [12], suggesting a system of compromised (but not lost) function. In fact, mRNA expression level does not track linearly with such deletions. It remains somewhat unclear if these monoallelic deletions do have a phenotype under genotoxic stress in human cells.

Mutations in homologous repair coordinating factors are often found in serous, clear cell, endometrioid, and carcinosarcoma ovarian cancers [47, 48]. Specific mutation patterns are found within BRCA1/2 or otherwise homologous repair deficient cells. Without functional homologous repair, cells default to non-homologous end joining (NHEJ) to repair double-stranded DNA (dsDNA) lesions. NHEJ does not perfectly repair DNA, but rather often introduces small insertions or deletions along with single-nucleotide variants at the break site. These mutational marks are frequently found in non-serous ovarian cancers, yet are unlikely the main drivers of SCNA instability in serous ovarian cancer. However, NHEJ factors involved in repairing unresolved dsDNA breaks across different chromosomes, or creating translocations and other complex rearrangements, are compromised in 40% *ex vivo* ovarian cancer isolates [13, 49]. This can lead to resistance to PARP inhibitors and promote inappropriate translocation or "repair" events [50]. Such defects in NHEJ may explain why the majority of SOC initially respond to cisplatin-based chemotherapy. Complex dsDNA lesions incurred by cisplatin target NHEJ and promote mitotic catastrophe [51].

Genetic and epigenetic changes alter *BRCA1* and the homologous repair pathways in SOC. Gene breakage is commonly observed within *RB1*, *NF1*, *RAD51B*, and *PTEN* [13]. While suppression of homologous recombination may lead to initial disease formation, there is evidence of *BRCA1/2* reversion mutations in tumors which become chemoresistant. This phenomenon follows the strong selective effects of carboplatin and taxanes which require cellular DNA repair pathways to enable cell division. Clinically, these findings should be considered in the context of the search for patient populations for PARP inhibitors. A primary hypothesis for how PARP inhibitors like Olaparib and Niraparib work is by targeting a DNA repair pathway which compensates for homologous repair, thereby presenting synthetic lethality specifically in cancer cells [52]. In a Phase III trial of Niraparib, clinicians treated both BRCA1 or BRCA2 mutant tumors as well as patients who were not found to have mutations in homologous repair genes in their tumors. Unexpectedly, all groups responded to Niraparib therapy [53], although patients with mutated BRCA1/2 or otherwise were defective in homologous repair were further delayed in cancer progression. While this is certainly an exciting development in the treatment possibilities for SOC patients, some caution is warranted. Reversion mutations enabling resistance to Niraparib may actually confer resistance to subsequent chemotherapeutics normally used upon disease recurrence [54].

Loss in BRCA1 enables microsatellite instability in mouse models and in colorectal cancer [55], though not in ovarian cancer [56, 57]. Microsatellite instability directly leads to centrosome

amplification, but SCNA instability, which may explain why it is observed in only a small minority of ovarian cancer patients, and is not linked to BRCA mutation status. Nonetheless, BRCA genes are inactivated through allelic deletions and expression modulation in ovarian cancer. Inactivation of *TP53* also suppresses BRCA1 expression [58]. BRCA1 interacts with ATM, directing it to phosphorylate p53 to enable p21 induction and G1/S phase cell cycle arrest [59, 60]. Without these complementary functions, tumors spontaneously form in BRCA1−/− p53+/− mice [61]. Centrosome duplication correlates with BRCA1 deletion [60]. This complex is thus a critical factor determining proper chromosome segregation during mitosis, and upon centrosome duplication aneuploidy is assured to form upon cell division [62, 63]. This is facilitated by prior mutations of p53 that abolish its checkpoint control function, again stressing the role for early mutation of TP53 in SOC.

Coincident SCNA events enable subsequent SCNA catastrophe. BRCA1 is located within kilobases of the neighboring autophagy gene BECN1. Autophagy is a critical catabolic infrastructure that enables cellular survival, requiring only 10% or less of normal autophagy gene dose [64]. Monoallelic loss of *BECN1* promotes centrosome amplification and aneuploidy among apoptosis-resistant cells [65]. An activator of the BECN1–PIK3C3 autophagy initiating complex, UVRAG, displays a similar phenotype [66]. Remarkably, this centrosome amplification promotes cell migration independent of its function in aneuploidy via indirect, hyperactivation of Rac1 [67]. LC3B, a ubiquitin-related protein which marks autophagosomal membranes, also acts in microtubule quality control [68]. One allele of the LC3B gene, *MAP1LC3B*, is genetically deleted in over 75% of SOC, independent of changes to *BRCA1* and *BECN1*. These three aneuploidy-accelerating lesions are likely to play key roles in serous ovarian cancer tumor initiation: mutant p53 expression, along with *BRCA1* and *BECN1* loss. Additional tumor suppressors may synergize to foster genomic instability: *NF1* is on the same chromosome arm as *BRCA1* and *RB1* lies nearby to *BRCA2*. Cumulative haploinsufficiency associated with prime targets and nearby neighbors certainly contribute to SOC aneuploidy [9] (**Figure 3**).

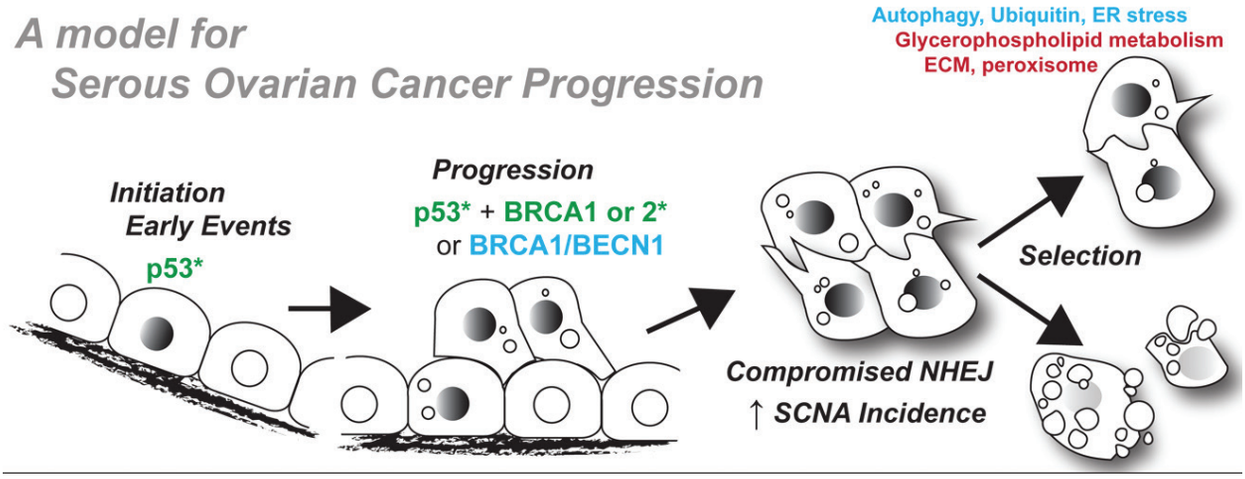

Figure 3. A model consistent with the ubiquitous mutation of p53 (SNV, indicated by green text) together with early SNV or haploid loss (indicated by light blue text) of repair enzymes, permitting a self-sustaining cascade of SCNAs and subsequent *in situ* selection for the observed SOC phenotypes.

In summary, *BRCA1/2* and homologous repair components are often suppressed by genetic deletions in SOC. This leads to further increases in SCNA formation and potentially independent metastatic phenotypes. However, SOC patients may benefit from the cancer's reliance on DNA repair pathways, as inhibition of PARPs prolongs progression free survival.

6. Pathways affected by SCNAs in serous ovarian cancer

Each cancer probably evolves at least 6–10 independent oncogene or tumor suppressor alterations [69] to circumvent natural homeostatic controls known as the "Hallmarks of Cancer" [70, 71]. These hallmarks include the evasion of regulated cell death, immortalization through telomere maintenance, defects in cell cycle control, immune system suppression, and enabling of metastatic capacity through physical and metabolic means. Traditionally, it has been assumed that single gene mutations are responsible for many of these oncogenic changes. Altered p53 function promotes escape to half of these hallmarks on its own, and mutations in strong oncogenes such as Ras family members, or growth factor receptor genes such as FGFRs, Her2 and even Met supplement many of the remaining hallmarks.

Individual gene amplifications can impact serous ovarian cancer. Aside from *TP53* and *BRCA1/2* mutations already discussed, tumors appear to be selected for specific chromosomal aberrations. Amplification of chromosomal region 8q, which contains the oncogenes *MYC* and *PTK2*, is a commonly found SCNV is SOC Myc overexpression promotes cell cycle progression, angiogenesis, and expression of target genes downstream of many other oncogenic factors such as NF-kB, β-catenin, and growth factor receptors [72]. Myc activation coordinately drives proliferation and promotes apoptosis, though since Myc-mediated apoptosis is p53-dependent, the pathway is averted. *PTK2*, the gene encoding focal adhesion kinase (FAK), enables metastatic phenotypes, cancer stem cell self-renewal, and neovascularization [73]. It is often co-amplified with *MYC*, as they both lie within cytoband 8q24. Myc overexpression is difficult to target therapeutically, although there are clinical trials underway for FAK inhibitors [74].

Recently, we analyzed single nucleotide and short 'in frame' deletion mutations across 120 validated oncogenes and tumor suppressors, finding that as many as 48% of serous ovarian primary tumors do not contain mutations in *any known* tumor suppressor or oncogene other than *TP53* [8]. Nonetheless, the average tumor has two-thirds of its genome altered by SCNAs. These findings support the notion that the actions of single oncogenes and tumor suppressors can only explain a portion of the genetics of ovarian cancer.

To analyze this, we developed new pathway network analytics tools to identify disrupted pathways in serous ovarian cancer in this unusually unstable genetic background. Despite the high levels of heterogeneity across patients, we found that coincident gene disruptions fell along surprisingly consistent patterns tumor-to-tumor, specifically suppressing or amplifying specific cellular pathways.

6.1. Autophagy

By far the most significantly suppressed pathway which stood out as unique in serous ovarian cancer and triple negative breast cancer was macroautophagy, which is most commonly

known, simply, as autophagy. The term autophagy ("*self-eating*") appropriately defines the process that cells use to recycle various macromolecular components, such as protein aggregates, lipids, and even entire organelles [75]. Autophagy is a primary method for the cellular catabolism, complementing turnover of proteins by the ubiquitin-proteosome system. Autophagy is described in terms of flux, which is the throughput of cellular '*detritus*' into autophagosomes, their transport to lysosomes, and subsequent enzymatic digestion. Ovarian cancer autophagy deletions impact the process primarily through deletions in *BECN1* (>75% of serous ovarian cancers) and in *MAP1LC3B* (>80% of serous ovarian cancers), though other genes are frequently affected. The average SOC tumor is 1 N across at least five different alleles; 95% of all serous ovarian cancers are deleted in BECN1 or *MAP1LC3B* and two others. The deficiency is as characteristic of SOC as p53 mutation. In addition to their roles in regulating chromosomal instability outlined above, *MAP1LC3B* and BECN1 (with the class three PI3 Kinase VPS34) play key roles in the formation of the early autophagosome, the phagophore, and recruitment autophagosome expansion proteins [76, 77].

Given this critical cellular function, we considered it counter-intuitive that cancer cells would delete a wide array of autophagy genes. In fact, KRAS mutant cancers have been described as "addicted" to autophagy, particularly in hypoxic or otherwise nutrient-stressed microenvironments [78]. This interpretation has been debated [79, 80], but the fact that autophagy has been established as a tumor suppressor system [81, 82], it is not exclusive of the possibility that specific tumor genotypes can promote addiction to autophagy [78]. Mono-allelic losses in the autophagy gene *BECN1* (homozygous deletions occur in only 0.9% of SOC cases) potentiate early development of tumors in mouse models [83, 84]. In this context, it is not at all counter-intuitive to consider that these gene losses likely synergize with defects in the BRCA1/2 pathway, the p53 pathway, and other initial SCNAs, thus producing the unique extreme level of aneuploidy associated with SOC. Moreover, the loss of gene copies does not completely "turn-off" autophagy. In fact, ovarian tumor cells, like other cells, require autophagy to provide clearing of protein aggregates, metabolic byproducts (especially in hypoxic environments), and possibly even to permit cell division, given their aneuploid state and relative chromosomal instability. This, in turn, may provide a second selection criteria for depressed autophagy. Autophagy is induced by missegregating chromosomes, chromosomal instability is a hallmark of SOC, and extreme induction of autophagy can promote cell death [85]. Therefore, it may be more appropriate to use the term "disrupted" rather than "suppressed" to define how ovarian cancer autophagy varies from that seen in normal somatic cells. The state renders SOC sensitive to agents that perturb autophagy by inhibiting the autophagic flux, or via the creation of proteotoxic stresses which must be resolved by autophagy (as discussed below).

6.2. Proteosome

Interestingly, a number of other proteostasis control pathways were suppressed in serous ovarian cancer, and foremost among these is complementary to autophagy, the ubiquitin-proteasome system. The core subunits, encoded by *PSMA1*, *PSMB1*, and *PSMC1*, are monoallelically deleted in 49, 62, and 41% of patients, respectively. Interestingly, the most interactive and deleted components of the proteasomal degradation pathway in ovarian cancer are enriched for cell cycle control related E3 ligases, including Park2, Fzr1, and Ube2d3. This suggests that not only is the core recycling process partly compromised by the core component

deletions, but that the pathway is redirected to allow for cell cycle progression proteins to persist and push the cell through division. The latter finding is perhaps to be expected, given that this has been established as a mechanism for tumor formation in many reviews [86–88]. Yet the proteasome may have a similar function to autophagy in suppressing aneuploidy. In a screen for mutations which are enabling for cell cycle progression in aneuploidy cells, ubiquitin-proteasomal degradation components were a top hit [89].

6.3. p53 Interactome

In addition to *TP53* gene mutation, serous ovarian cancer exhibits a number of p53-interacting components that are also suppressed by deletions. Among the top hits by HAPTRIG [8] include *CHEK2*, *BAX*, and *GADD45A/B* gene deletions, along with *CCNE1* and *ATR* amplification. Chk2 is a kinase which coordinates DNA repair and cell cycle arrest, in part by stabilizing p53. Bax is a pro-apoptotic Bcl-2 family member which associates with p53 to induce apoptosis [90]. The Gadd45 proteins mediate DNA damage signaling to p53 and act as tumor suppressors by leading to damage-induced senescence [91]. Conversely, an upregulated ATR network allows for potential enhancement of DNA repair pathways which lead to aneuploidy and may also lead to centrosome duplications [92]. This is further supported by a common suppression of Rad51 networks in SOC.

6.4. Metabolism

Metabolism is fundamentally disrupted in serous ovarian cancer. This may be predicted by the observation that patients with metabolic disruptions are at risk for disease, or have a predisposition to tumors to undergo metastatic growth to adipose tissue [93, 94]. A shift to glycolysis, the Warburg effect, is a general hallmark of cancer. Glycolytic shift is considered essential to provide the many constituent molecules required for cell division: nucleotides, lipids, and amino acids, moreso than simply ATP which is produced in higher quantities by oxidative phosphorylation [95]. A metabolic pathway found to be suppressed with almost equal magnitude to autophagy was the arginine and proline metabolism pathway, particularly through deletions in *SAT1* and *SAT2* and guanidinoacetate N-methyltransferase. Such deletions are predicted to reduce spermidine metabolism and polyamine formation, which is normally upregulated in tumors [96]. The reason for their ubiquitous suppression may lie in the increase in glutamate which would come from an inhibition of arginine biosynthesis, which can then be used in the TCA cycle [97].

6.5. Adipocytokine

Adipocytokine signaling and *fatty acid* metabolism was also altered, led by suppressed networks with the *CPT1B* gene and *ADH4,6,7*, and *1A*. Again, this result is unique and unexpected: *CPT1* isoforms are often upregulated in prostate cancer [98] as are ADH enzymes [99]. Dysregulation of ADH isoforms may enable acetaldehyde formation, which is oncogenic, or favor class I alcohol dehydrogenases, which are upregulated in cancerous ovarian tissue [100]. Conversely, one of the most upregulated metabolic pathways in serous ovarian cancer is glycerolipid metabolism. Upregulation is led by amplification of the *DGAT1* gene, encoding diglyceride acyltransferase, the committing step for synthesis of triglycerides and

an essential reaction for the formation of adipose tissue. The pathway is further reinforced by overexpression of *LPIN1* and *LPIN3* genes. While targeting metabolism has not historically been successful in cancer treatment, overexpression of these genes may act as early identifiers of ovarian cancer.

6.6. Peroxisome

An unusually altered pathway in serous ovarian cancer bridges metabolism, fatty acid oxidation and proteostasis disruption: *peroxisome* biogenesis. Peroxisomes are subcellular organelles whose primary function is to metabolize reactive-oxygen species and provide lipids to other organelles [101]. This pathway is amplified in serous ovarian cancer, and lung adenocarcinoma only [8]. *PEX5*, *PEX5L*, and *PEX19* are all commonly amplified. Pex5 and Pex19 bind to peroxisome enzymes in the cytosol and direct them to the peroxisome matrix [102, 103]. In fact, amplification of PEX5 is associated with poorer outcome in SOC. Peroxiredoxin-1 is also strongly amplified, and can be detected at increased levels in ovarian cancer patients' serum [104], and is also associated with lung cancer malignancy [105]. Upregulation of this pathway, and those associated with phospholipid metabolism may provide to a means to overcome oxidative stress, perform fatty acid beta-oxidation, and resist lipotoxicity associated with invasion of adipocyte-rich regions of the omentum.

While each of these pathways can help to define phenotypes associated with SOC, they also have the capacity to enable development of new classes of pathway-targeted therapeutics. It may be possible in future for SCNA-modified pathways to serve as targets the same way that SNVs do, now.

7. Potential for new treatments by targeting copy number alterations

SNVs have a proven track record of targetability using small molecules. Nonetheless, in the case of SOC, new cures are unlikely to be found unless somatic copy number alterations (SCNAs) are considered. Defining this interplay will be a difficult task. It remains unclear exactly which SCNAs are most critical to SOC proliferation and metastasis. The creation of cell line models will require new methods of whole-chromosome manipulation, even as attracting pharmaceutical company support will be harder due to limited experience which such targeting strategies, as well as conservative business approaches towards eventual clinical adaptation. Nonetheless, there are reasons to be optimistic that SCNA-targeted therapeutics can be effective and that some could enter the clinic in the near future.

Consider the abundance of SCNAs in advanced SOC relative to other cancer. The successful tumors have undergone selection. The phenotypes produced include well-known hallmarks of cancer: including cell cycle defects, heightened glucose uptake [106], spontaneous proliferative immortality [107], and dysregulated autophagy [108]. The same studies identify aneuploidy-associated characteristics which present vulnerabilities particular to these unstable cells. Perhaps the most promising vulnerability is an increased reliance on protein quality control processes such as ribosome biogenesis and maintenance factors and the cellular recycling process, autophagy. Aneuploid cells require these systems to function, and may result in a general

reliance on catabolic function due to the proteotoxic effect of protein-complex subunit imbalance. Early studies recognized a general, if partial, sensitivity of these cells to rapamycin [106].

Chromosome instability can incur resistance to taxanes, a common front line therapeutic for SOC [109]. Chromosomal instability endowed by docetaxel may in fact lead to subsequent additional chemoresistance [110], though it is clear that the resistant phenotype is at least initially offset by an increased sensitivity to carboplatin, the second primary chemotherapeutic co-administered with a taxane as standard of care. Although aneuploidy enables oncogenic characteristics, it offers targetable vulnerabilities as well.

The mapped SCNA patterns in SOC revealed a general fault in proteostasis control, centered on autophagy [8]. Yet these cells require autophagy to maintain viability. The delicate balance within SOC relative to normal tissue, appears to provide a therapeutic window for proteostasis-targeting agents. Since SOC cells are already severely disrupted in their proteostasis-regulatory mechanisms, further disturbance can greatly compromise survival even as normal cells readily process the insult. Given this premise, we developed the Combination of Autophagy Selective Therapeutics (COAST) method to effectively manage SOC in the lab [8]. The general approach involves directly stressing the proteostasis system, while inhibiting autophagy resolution (**Figure 4**).

Mice given the cocktail of five proteostasis drugs did not lose weight nor negatively alter their blood chemistry panel [111], tolerating these drugs for months of daily treatment [8]. In mouse models using recurrent human SOC cells, the proteostasis drugs out-performed platinum-taxane dual treatment. The results are consistent with previous approaches pursuing a "cyclops" hypothesis: that monoallelic deletions in cancer sensitize cancer cells to further disruption of that gene's function [112]. As normal cells bear a full complement of

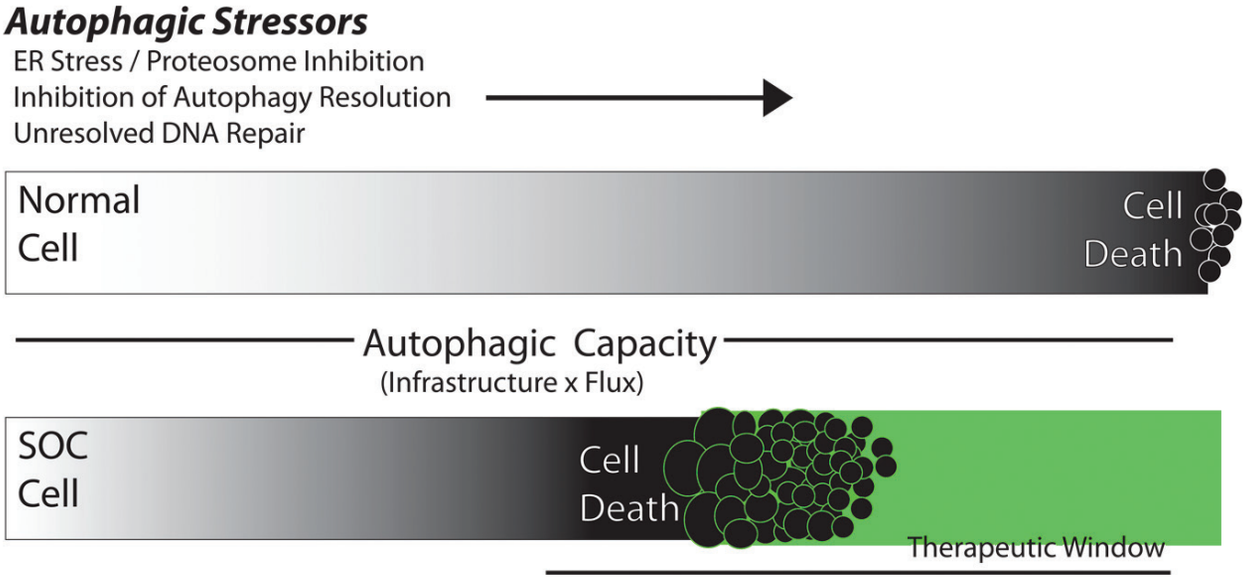

Figure 4. SCNA can compromise key cellular infrastructure. The figure shows how the disruption of autophagy capacity that is observed in SOC can render the cells more sensitive to agents that modulate autophagy. The lack of genetic infrastructure, combined with a constant requirement for autophagy, opens a therapeutic window for these agents. Nonetheless, the heterogeneity within tumors and between patients insures that no single agent, used alone, will provide a complete benefit.

all pathway genes, they are typically less sensitive to such stresses, which opens therapeutic windows for treatment.

Given that proteostasis pathways are only one type of disruption caused by SCNAs in SOC, what other SCNA-disrupted pathways might be targetable? Alluring targets may include the proteins downstream of the E3 ligases commonly deleted in SOC; inhibition of cell-cycle regulators may selectively target SOC cells even without any mutation or than copy number changes. Strong amplification of peroxisome transporters and the glycerophospholipid metabolism pathways suggest that metabolic targeting may be worthwhile. *GSK3B* controls signaling between varying development and stem cells pathways [113], and was marked as the most cumulatively impactful amplified gene in SOC by HAPTRIG [8]. Interestingly, inhibition of GSK3β in preclinical models of SOC showed strong tumor inhibition [114], and a number of drugs targeting GSK3β are in development for cancer, diabetes, and neurodegeneration [115]. Finally, the 8q24 region is the most commonly amplified genomic region in SOC, containing MYC and FAK. Inhibitors to FAK, such as defactinib, are already being tested in the clinic [116], and will hopefully provide some positive results in the near future.

A caveat of such designs is heterogeneity inherent in disease. Copy number instability is the result of SOC cells' extraordinary ability to create, tolerate, and expand genomic variation. Mathematical modeling of real tumor genetic data suggests that even small tumors with low mutation rates are statistically likely to contain multiple independent clones able to resist a particular drug treatment [117, 118]. Current SOC chemotherapeutics stimulate aneuploidy. Taxanes result in chromosome missegregation and platinum agents promote translocation events due to cross-strand DNA lesions. While it is absolutely true that common chemotherapeutics have limited combination potential due to dose limiting toxicities, that does not preclude the use of highly specific drugs to be used in combination or as maintenance therapy in SOC treatment regimens. Most likely, drugs independently targeting the many SCNA-disrupted pathways may be required to completely cure a patient.

While most patients are caught late in the evolution of their disease, it may not be "too late" to treat them. A genomic analysis in highly metastatic recurrent SOC patients found that the tumors likely form a metastasis-to-metastasis spread [14]. This may explain why round after round of different chemotherapy can extend the life of SOC patients [119]. This implies that current chemotherapy is quite effective at destroying a great majority of cells, and the challenge that remains is how to complement it. The COAST strategy studied in our lab functioned equally well or better for cisplatin resistant forms of SOC [8]. Autophagy has been widely implicated in the ability of quiescent cells to survive, including in SOC [120], and has been directly shown to enable growth of Doxil resistant disease [121]. However, given that one COAST agent, chloroquine is often prescribed for the same patient for decades in high-risk malaria areas, while another, nelfinavir, is a daily long-term HIV medication with no serious side-effects, the use of COAST is warranted based on the decades of use of COAST drugs, in humans, for diseases other than cancer. The side effects are well established to be below current chemotherapeutics carboplatin, paclitaxel, and Doxil [111]. The greatest health concern may lie in kidney cells, which are also exquisitely sensitive to autophagy drugs [122].

Since the drugs target different but complementary pathways, it is feasible to design clinical trials involving either simultaneous treatment or sequential treatment, enabling a greater

chance of minimized side effects. Compromised DNA repair feeds into this pathway, suggesting that the recent successes of the PARP inhibitors are not simply due to BRCA1 complementation. An expanded range of options must be aggressively explored in the near future if we are to understand how to exploit the SCNA genetics of ovarian cancer in a timely fashion.

Abbreviations

AOC	Australian Ovarian Cancer (study)
N	the number of copies of a given gene present in a cell (e.g., 3N)
SCNA	somatic copy number alteration
SNV	single nucleotide variant (a point mutation)
SOC	serous ovarian carcinoma
TCGA	the Cancer Genome Atlas
TP53	tumor protein 53 kDa gene (protein is p53)

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