

NEWS AND COMMENTARY

Ovarian Cancer

Identification of remodeling and spacing factor 1 (*rsf-1*, *HBXAP*) at chromosome 11q13 as a putative oncogene in ovarian cancer

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Overall survival for epithelial ovarian cancer patients has not improved significantly in the past 20 years, indicating that an improved understanding of the genomic events underlying the initiation and progression of ovarian cancer is greatly needed. Like most solid tumors, ovarian cancer has a high degree of chromosomal instability, and both total and regional instability are associated with altered patient outcomes.¹

An identification of the 'drivers' of genomic aberrations could greatly improve our understanding of ovarian cancer and contribute to improved patient management. We and others have identified many such potential 'drivers', including *Rab25* (1q22), *PIK3CA*, *evi1*, *protein kinase C iota*, *SnoN* (3q26.2), *myc* (8q24.2), *HER2* (17q12), *AKT2* (19q13.2), and *ZNF217* and *EEF1A2* (20q13.2).^{2–7} These candidate drivers have been reported to be aberrant at the DNA, RNA and protein levels, to alter the phenotypic behavior of tumor cells, and to correlate with patient outcomes.

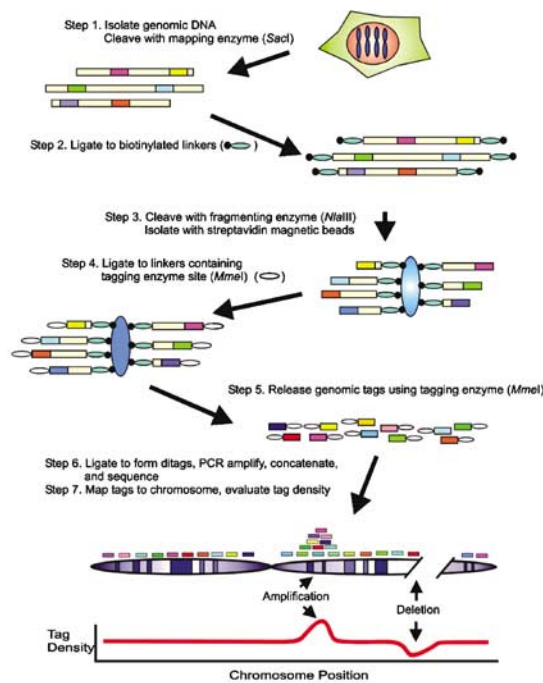
In a recent issue of PNAS, Shih *et al*⁸ provided evidence at the DNA, RNA, protein, and functional levels that *Rsf-1* played a major role in the development or selection of the 11q13 amplicon in ~13–17% of ovarian cancers. Importantly, elevated levels of *Rsf-1* correlated with worsened patient outcomes, suggesting that it may have use as a marker of tumor behavior and furthermore that manipu-

lating *Rsf-1* levels/function could result in improved outcomes. This study implicated functions of *Rsf-1*, including chromatin remodeling and gene transcription, in the pathophysiology of ovarian cancer.

Shih *et al*⁸ used digital karyotyping to identify and map genome wide copy number changes in ovarian cancer at high resolution. This approach involved the isolation and enumeration of short sequence tags from specific genomic loci (Figure 1).⁹ Genomic DNA was cleaved into several hundred thousand pieces with a restriction endonuclease (mapping enzyme). Biotinylated linkers were ligated to DNA and digested with a second endonuclease (fragmenting enzyme) that recognized 4-bp sequences. DNA fragments containing biotinylated linkers were separated from the remaining fragments using streptavidin-coated magnetic beads, and new linkers, containing a 6-bp site recognized by the *MmeI* type IIS restriction endonuclease, were ligated to the captured DNA. The captured fragments were cleaved by *MmeI*, releasing 21-bp tags. Isolated tags were self-ligated to form ditags, polymerase chain reaction (PCR) amplified, concatenated, cloned, and sequenced. Finally, tags were computationally extracted from sequence data, matched to precise chromosomal locations, and tag densities evaluated over moving windows to detect abnormalities in DNA copy number.

Shih *et al* mapped a previously identified chromosome 11q13.5 amplicon in two high-grade ovarian carcinomas and the OVCAR3 cell line to a minimal 1.8 mb region containing 13 genes. They used quantitative real-time PCR and dual-color fluorescence *in situ* hybridization to confirm their finding and to demonstrate 11q13.5 amplification in 16/121 (13.2%) high-grade serous carcinomas. *EMSY*, which had been previously implicated in the 11q13.5 amplicon in breast and ovarian cancers^{5,6} was amplified in 12/121 patients. Of the 13 genes, *Rsf-1* (*HBXAP*) demonstrated consistent over-expression in 11q13.5-amplified tumors as well as marked differences between amplified and nonamplified specimens. In contrast, *EMSY* was coamplified in 8/121 samples but its mRNA level was not consistently upregulated in amplified tumors. It is important to note that *EMSY* was amplified in four tumors without *Rsf-1* amplification. There was a statistically significant correlation between *Rsf-1* gene amplification and *Rsf-1* protein expression, further supporting *Rsf-1* as a driver of the 11q13.5 amplicon.

The 11q13.5 amplicon, which is present in a fraction of ovarian, breast, and head and neck carcinomas, has been proposed to contain a number of potential candidate drivers, including *CCND1*, *FGF4/3*, *EMS1*, *GARP*, *PAK1* and *EMSY*, centromeric to *Rsf-1*, and *CLNS1A*, *ALG8*, and *GAB2* near *Rsf-1*. The role of *PAK1* in important signaling pathways and the interaction of *EMSY* with *BRC A2* support a role for these genes as candidate drivers.⁶ *CCND1* is centromeric to the minimal 1.8-Mb region of amplification involving *Rsf-1* at chr11:77 054 922–77 092 226 in ovarian cancer but may, however, be located in the 11q13 amplicon in other tumors. The development and selection of amplicon structure in ovarian cancer is likely to be complex. For example, multiple different drivers probably exist for the 50 mb 3q26 amplicon in ovarian cancer. Further, the genes within this amplicon may function in concert during development and selection of amplicon structure. Thus, it is not necessary that a single gene functions as the 'driver' of amplification. Indeed, while this study presents strong evidence supporting *Rsf-1* and nearby genes including *CLNS1A*, *ALG8*, and *GAB2* as potential



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Figure 1 Schematic of the digital karyotyping approach. Colored boxes represent genomic tags. Small ovals represent linkers. Large blue ovals represent streptavidin-coated magnetic beads (From reference 9 with permission).

drivers of the 11q13.5 amplicon, it does not eliminate a contribution of other close genes such as *PAK1* or *EMSY* in a subset of patients. Indeed, the amplicon at 11q13.5 in a number of ovarian cancer patients does not include *Rsf-1*.

Rsf-1 and its binding partner, *hSNF2H*, contribute to chromatin remodeling, transcriptional regulation, cell cycle progression, and carcinogenesis.^{10–15} Shih *et al* postulated that *Rsf-1* overexpression in tumor cells could disrupt homeostatic kinetics in chromatin remodeling, thereby facilitating tumorigenesis. As *Rsf-1* appears to be selectively amplified and overexpressed in late-stage tumors, it likely plays its major role during tumor progression. However, it remains possible that *Rsf-1* is functionally disrupted in early tumors in a manner not detected in this study.

We are still limited by poor understanding of the molecular mechanisms underlying the development and selection of genomic amplifications and deletions in

cancer. The frequency of genomic aberrations, their correlation with patient outcomes, the location of important genes within the aberrations, and a high degree of correlation between DNA copy number and RNA and protein levels suggests that these aberrations play a role in the initiation or progression of epithelial tumors. However, it remains possible that the genomic aberrations are secondary to underlying genetic instability or indicative of a more complex process. For example, in some lung cancers, the initiating event may be mutational activation of the epidermal growth factor receptor gene followed by selective amplification. Nevertheless, these genomic aberrations have directed attention to a number of putative oncogenes and tumor suppressor genes. Although manipulation of many of the candidate genes in particular amplicons alters phenotypic outcomes in ovarian and other cancers, it is still not clear whether this observation simply reflects the importance of human genes or a more

selective effect of the gene in driving genomic amplification. Direct tests of the role of potential drivers in the development of amplicons will likely require demonstration of the effect of the candidate gene on development or structure of the amplicon during tumor development in cell line or animal models. Thus, an improved ability to combine information from global genomic, transcriptional, and proteomic technologies to identify and characterize cooperating events between multiple genes and genetic aberrations will be necessary to deal with the complexity of tumor development ■

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