with regard to the precise localization of DC subsets within the tumor bed. Broz et al. (2014) found that both CD11b$^+$ DC1 and CD103$^+$ DC2 were preferentially located in collagen-rich zones distal to the tumor nodules where TAM1 and TAM2 cells were found. In contrast, Ruffell et al. (2014) report that CD103$^+$ cells were dispersed throughout the tumor stroma in the proximity of macrophages. Although Ruffell et al. (2014) found no change in the localization of CD103$^+$ cells after treatment with paclitaxel and αCSF-1, Ma et al. (2013) found that CD11b$^+$ cells exhibited a selective tropism for dying tumor cells after doxorubicin treatment.

Irrespective of these discrepancies, however, the accumulating evidence suggests that some DC subpopulations can cross-present tumor antigens within the cancer without needing to migrate to lymph nodes. Thus, lymphadenectomy fails to affect the anticancer immune response elicited by anthracycline-based chemotherapy (Ma et al., 2013, 2014). Moreover, direct purification of intratumoral DC subsets yields functional tumor antigen-presenting cells that are able to prime naive T cells in vitro (Broz et al., 2014) and elicit anticancer immune response upon adoptive transfer in vivo (Ma et al., 2013). These results reinforce the idea that the tumor may be considered as a full-blown lymphoid organ, in which all steps of cellular immune responses starting with appropriate presentation of tumor antigens by dendritic cells occur in situ.

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Building through Breaking: The Development of Cancer Neochromosomes

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In this issue of *Cancer Cell*, Garsed and colleagues combine chromosome flow sorting and deep sequencing to characterize the structure of oncogene-containing neochromosomes in liposarcoma and provide evidence that they are generated by a combination of multiple dynamic and destructive processes.

Loss of genomic integrity in cancer has many different manifestations. In this issue of *Cancer Cell*, Garsed et al. (2014) investigate one of the most convoluted products of this genomic instability—the neochromosome, characteristic of well-differentiated/dedifferentiated liposarcoma (WD/DDLPS). The term "neochromosome" describes a marker chromosome whose origin cannot be determined by conventional chromosome banding techniques, which emphasizes their extreme divergence in size and structure from any normal chromosome. By combining chromosome flow sorting and deep sequencing, Garsed et al. (2014) characterize the structure of these remarkably large and highly rearranged structures and propose a model for their genesis and growth. Undergoing multiple rounds of such catastrophic events as chromothripsis, breakage-fusion-bridge cycles, and centromere erosion, their survival is a testament to the power of selection and the ability of tumors to leverage destructive processes for their own benefit.

The heterogeneous collection of malignant tumors of adipose tissue known as liposarcomas constitutes roughly 20% of all sarcomas (Dei Tos, 2014). The most common subtype of liposarcoma, accounting for nearly half of all cases, is the WD/DDLPS, also referred to as atypical lipomatous tumor in some circumstances. Although the higher grade DDLPS is metastatic in roughly 20% of cases, the primary difficulty of this malignancy is associated with local aggressiveness and recurrence. Early cytogenetic studies

of WD/DDLPS tumors revealed the striking, consistent presence of ring or giant marker chromosomes (Dei Tos, 2014), a feature now recognized as characteristic of the disease. These neochromosomes were subsequently shown to contain high-level amplifications of chromosome 12 region q13-q15 (Dei Tos, 2014), and further mapping pointed to the proto-oncogenes *MDM2* and *CDK4* (Berner et al., 1996; Nilbert et al., 1994). Mdm2 inhibits p53-mediated apoptosis and arrest while Cdk4 phosphorylation of Rb1 blocks its interaction with E2F transcription factors governing the G1-S checkpoint. Thus, this co-amplification provides a selective benefit by coordinating increased survival and proliferation.

While the selective benefit of amplifying 12q13-15 may appear straightforward, the exact structure of this neochromosome and the mechanism by which it develops has remained a mystery. The WD/DDLPS neochromosomes contain so much genetic material that they are, in fact, the largest chromosomes in the tumor cells. Garsed et al. (2014) begin by leveraging this to separate the neochromosomes from the rest of the chromosome complement by flow-sorting multiple WD/DDLPS cell lines. Consistent with the neochromosome being the most remarkable cytogenetic finding in such tumors, copy number analysis shows that nearly all of the amplified genetic material in the cell occurs on the neochromosome. Garsed et al. (2014) then use high-depth paired-end sequencing of the enriched neochromosomes to identify how they are stitched together. While each neochromosome is unique, they share several features. First, they each contain a "core" of highly, but unequally, amplified material from hundreds of genomic loci. Across cell lines, Garsed et al. (2014) found that the median length of the donor sequence is 23 Mb, which is amplified on average 10-fold, with some loci amplified considerably more. While each neochromosome incorporates and amplifies many different genomic regions, the only shared sequence across all the neochromosomes is 1.4 Mb from chromosome 12, which includes *MDM2*, *CDK4*, and several other genes.

Garsed et al. (2014) proceed to utilize the information in paired-end sequencing both to reveal the composition as well as to model the temporal ordering of structural rearrangements (Greenman et al., 2012; Sanborn et al., 2013) in the WD/DDLPS neochromosomes. The life history that emerges from these WD/DDLPS neochromosomes is apparently a tale of disaster upon disaster; it is quite surprising that anything functional, let alone beneficial for the cell, can be so created. The initial episomal structure appears to have been generated by stitching together some of the donor loci, including the chromosome 12 regions, in a nonamplifying process highly reminiscent of chromothripsis (Stephens et al., 2011).

The inferred double minutes apparently continue to receive donor sequences through additional chromothripsis, but the second stage of their development is dominated by progressive amplification and deletion of material through breakage-fusion-bridge cycles. While generation of double minutes by chromothripsis has been demonstrated previously (Sanborn et al., 2013; Stephens et al., 2011), Garsed et al.'s model for assembly and amplification of the initial episomal structures to the much larger neochromosome is novel. While typical double minutes do not contain centromeres and sort randomly to daughter cells at division, the centromere biology of these evolving WD/DDLPS neochromosomes is apparently quite dynamic. Standard alphoid centromeres are acquired but then degraded and lost, while neocentromeres are established at other loci, including across rearrangement junctions on the neochromosomes.

The final, stabilizing event for the neochromosomes appears to be linearization and telomere acquisition. While both circular and linear forms of the neochromosomes are often found in WD/DDLPS tumors, these cell lines contain only linear forms. Linearization appears to be a late event and is accomplished with the acquisition, but not amplification, of large telomeric fragments from diverse donor chromosomes. These additional sequences extend the length of the neochromosomes by over 100 Mb.

Garsed et al. (2014) provide a compelling description of the architecture of these neochromosomes, and the proposed model of their genesis and development is also highly consistent with the sequence data. Unfortunately, there is no experimental model in which the process of 12q-containing neochromosome development can be studied in living cells, and the scope of neochromosome sequencing is limited to relatively few cell culture sources. Therefore, of necessity, the exact nature of the earliest events in this process remains inferential. However, the generality of the structural model presented by Garsed et al. (2014) could be tested by whole genome sequencing of tumor DNA samples if it becomes practical to assemble chromosomes de novo with the development of improved long read sequencing technologies. Other questions also remain. How is chromothripsis initiated, and why is neochromosome formation so strongly associated with certain types of cancer? *MDM2* and *CDK4* amplification as double minutes or recognizable insertions in other chromosomes occurs in many different types of cancer, but 12q-containing neochromosomes are much more restricted. Intriguingly, they are particularly enriched in malignant, but typically less metastatic, sarcomas such as WD/DDLPS and parosteal osteosarcoma (Örndal et al., 1992). What favors assembly of chromosome fragments into large structures specifically in these histotypes, and precisely how does this occur? Is there an underlying susceptibility to this type of genomic instability in the mesenchymal lineage that gives rise to these tumors that could be defined biochemically (Crasta et al., 2012)? Conversely, do these cells experience an elevated selective benefit for this type of structure? The inactivation of p53 prior to catastrophic genomic processes such as chromothripsis has been demonstrated before (Rausch et al., 2012). In these cases, it is thought that the initial *TP53* mutation either facilitates the initiation of or survival after chromothripsis. In WD/DDLPS, however, it appears that chromothripsis precedes and, in fact, leads to p53 inhibition. Finally, if the process of neochromosome formation is so turbulent and dynamic, why are the other chromosomes relatively spared? Is the specificity for chromosome 12 completely explained by selection for 12q genes, or are there structural factors that confer a predilection to this process? The thought-provoking study by Garsed et al. (2014) has made significant inroads into the long-standing problem of the chromosome mechanics underlying 12q-amplification in WD/DDLPS and will certainly stimulate additional investigation of this remarkable phenomenon.

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ChIP-ping Away at EWS/ETS Transcription Networks

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In this issue of *Cancer Cell*, Riggi and colleagues use a genomic approach to define two distinct molecular mechanisms through which the chimeric EWS/FLI1 oncoprotein regulates target genes in Ewing sarcoma, expanding a framework upon which to model the target gene network and test strategies for antagonizing growth of this tumor.

Twenty two years ago, Delattre and Thomas pried open the door on Ewing sarcoma biology by reporting that the t(11,22) found in the large majority of these tumors created a fusion gene between EWS and FLI1 (Delattre et al., 1992). Finally, there was an unambiguous biomarker for a malignancy that, up until then, had been diagnosed primarily by exclusion. Of even greater importance was the prevalent belief that a somatic mutation that was present in 85% of Ewing tumors must be playing crucial oncogenic roles. This contention was quickly born out. Not only could ectopic expression of EWS/FLI1 avidly transform cells, but inhibition of the fusion consistently induced growth arrest of Ewing sarcoma tumor derived cell lines. Who could ask for a better therapeutic target for this deadly disease?

And then things got difficult. Structural function analyses of EWS/FLI1 indicated that the fusion was mediating its biologic effects by acting as an aberrant transcription factor. However, the portion of EWS that was fused to FLI1 was found to be unstructured, making it poor bait for protein interaction screens and a difficult target to develop small molecule antagonists against (Ng et al., 2007). EWS/FLI1, like many of the oncogenic chimeric fusions found in other sarcomas, earned the "undruggable" label. The fact that in the last two decades there has been only one candidate small molecule that specifically targets the EWS/FLI1 fusion, suggests that, at least so far, this reputation is deserved (Barber-Rotenberg et al., 2012).

So, if directly antagonizing EWS/FLI1 seemed unfeasible, perhaps targeting genes that were transcriptionally modulated by the fusion would be a therapeutically more tractable strategy. As molecular methods advanced, the number of EWS/FLI1 target genes that were identified geometrically increased. The problem was that demonstrating biologic relevance of these candidates proved to be labor intensive, unpredictable, and incomplete. In general, forced expression of any single EWS/FLI1 target gene did not recapitulate the EWS/FLI1 phenotype in cells. Conversely, target gene inhibition frequently did not completely shut down the cell transformation effects of EWS/FLI1.

This suggested that EWS/FLI1 transformed cells through the cumulative effect of transcriptionally modulating a network of genes. Inherent in such systems is a central robustness that can tolerate loss of one or more nodes while still maintaining the overall effect of the network (Friedman and Perrimon, 2007). As success in the search for EWS/FLI1's Achilles heel was proving progressively more unlikely, the need to generate a comprehensive map of the target gene network became more urgent. Although earlier work provided snapshots of this network, it was difficult to paste the pictures together into a coherent whole. What was needed was a wide-angle lens through which to view the broad EWS/FLI1 target gene network.