Of note, the two studies identify different Eph receptors as key in CSC maintenance, although some level of crosstalk likely exists between the Eph receptors as well as other RTKs central to maintenance of the hierarchy. It would seem this difference could not be due to differential representation within the recently identified GBM subclasses as both have highest expression in the mesenchymal and classical groups (Verhaak et al., 2010). However, Eph receptors may be informative within these subgroups, although that hypothesis would require further exploration. Importantly, both groups validate the efficacy of targeting Eph receptors in preclinical models.

In conclusion, these two reports are not simply additions of new CSC markers but rather help reinforce expanding opportunities for integrating features of normal tissue hierarchies and instructive microenvironmental cues in tumor development and maintenance that can inform advances in diagnosis and therapy.

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Breaking News on Fragile Sites in Cancer

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Chromosome rearrangements in B lymphocytes can be initiated by AID-associated double strand breaks (DSBs), with others arising by unclear mechanisms. A recent study by Barlow and colleagues in *Cell* reports on genomic regions, termed early replicating fragile sites, that may explain many AID-independent DSBs and creates a compelling link between replication stress, transcription, and chromosome rearrangements.

Recurrent chromosomal translocations are common features of many cancers, especially lymphomas and leukemias. Most appear to be formed by the joining of two double strand breaks (DSBs). In developing B cells, DSBs are introduced into immunoglobulin loci during V(D)J recombination and class-switch recombination (CSR). Both CSR and immunoglobulin somatic hypermutation are initiated by AID, a single-strand-specific DNA cytidine deaminase targeted to DNA by transcription (Nussenzweig and Nussenzweig, 2010). AID-associated DSBs often generate one of the two breakpoints in the translocations observed in lymphoid tumors. This programmed DNA damage also puts the lymphocyte genome at risk for rearrangements with bystander loci, such as the *C-MYC* locus. Nonetheless, while many translocations are driven by off-target AID-induced DSBs, others result from poorly defined factors that might include replication errors, oxidative stress, genotoxic agents, and involvement of chromosome fragile sites.

Common fragile sites (CFSs) have been recognized for decades as hotspots for breaks occurring on metaphase chromosomes following replication stress (Durkin and Glover, 2007). Following low doses of the DNA polymerase inhibitor aphidicolin (APH), chromosome breaks can be seen at discrete genomic regions that span hundreds of kilobases, often in large genes. CFS instability is dependent on ATR signaling and associated with other DNA damage response factors (Durkin and Glover, 2007). Le Beau et al. (1998) and studies that followed showed that CFSs replicate late in S-phase and sometimes escape to metaphase with incomplete replication. For decades, two nonexclusive models have existed for CFS instability. One is that CFSs contain difficult-to-replicate sequences, leading to stalled replication forks. The second is that CFSs contain a paucity of replication origins, leading to late or incomplete replication. Support for the former came from the fact that CFSs are AT-rich and contain a high number of "flexibility peaks" (Zlotorynski et al., 2003) capable of forming secondary structures, especially when

replication is perturbed, that can act as barriers to replication. Recent experiments (Letessier et al., 2011) further provided evidence for a paucity of active origins at some CFSs, reflecting a failure to activate dormant origins in these regions following replication fork arrest. Importantly, replication and fragility patterns both differed among cell types.

Since their discovery, attempts have been made to link CFSs to translocations and other rearrangements in cancers. Whereas positive correlations were found at the chromosomal level, most did not stand up to higher resolution inspection. Nonetheless, CFS instability has been associated with gene amplifications and a small number of translocations in cancers (Arlt et al., 2006). Recently, replication stress induced by APH or hydroxyurea (HU) has been shown to be a potent inducer of submicroscopic deletions and duplications, i.e., copy number variants (CNVs), with some CNV hotspots at CFSs (Arlt et al., 2011). These CNVs can span hundreds of kilobases and model many CNVs that arise frequently in cancer cells and in the human germline.

Recently in *Cell*, Barlow et al. (2013) opened a new chapter in the fragile site and cancer saga that has important implications for cancer risk. The authors identified genome-wide early-firing replication origins, sites of RPA binding indicative of ssDNA accumulation and sites of active transcription in mouse splenic B cells after release from G1/S arrest induced with high doses of HU. Comparison of the data sets revealed a highly significant overlap. Thus, HU-induced RPA recruitment in early S phase preferentially occurred at early origins of actively transcribed genes. The sites were marked by γH2AX binding and were associated with BRCA1 and SMC5, indicative of replication fork collapse and DNA damage response activation. The authors termed these sites "early replicating fragile sites" (ERFSs) because their analysis focused on the beginning of S phase, in contrast to the late replication associated with CFSs. Consistent with this difference, ERFSs arose at different genomic loci than previously mapped CFSs.

To investigate if ERFSs are prone to chromosome breaks like CFSs, they treated cells with high-dose HU and examined metaphases with FISH probes to ERFS hotspots. All six ERFS hotspots displayed CFS-like chromosomal breaks with 8%–15% of total damage at these six loci. Like CFSs, ATR inhibition and oncogene stress promoted ERFS breakage, consistent with the arrested replication and ssDNA observed at these sites. By studying breakage at an ERFS near *SWAP70* in cell types with different levels of transcription, the authors found a positive correlation between fragility and transcriptional activity despite similar replication timing, supporting a mechanistic link. Comparison of wild-type and AID knockout B cells demonstrated that ERFS fragility is AID independent.

How do ERFSs relate to chromosome rearrangements in cancer? To address this, the authors examined three ERFSs, including one in the lymphoma-associated *BACH2* locus, in metaphases from AID-overexpressing, 53BP1-deficient B cells, which have G1 *IgH* breaks that persist into S phase where they might be joined to ERFSs. They found chromosome breaks at both the *IgH* and *BACH2* loci. Furthermore, *BACH2* translocations to unidentified chromosomes were found in 1.2% of metaphases and to *IgH* in one cell. They then compared ERFSs with copy number alterations (i.e., CNVs) detected in biopsies of diffuse large B cell lymphoma, the most common non-Hodgkin's lymphoma. Strikingly, 51.6% of the 190 common amplifications and deletions in the patient samples overlapped with ERFS regions.

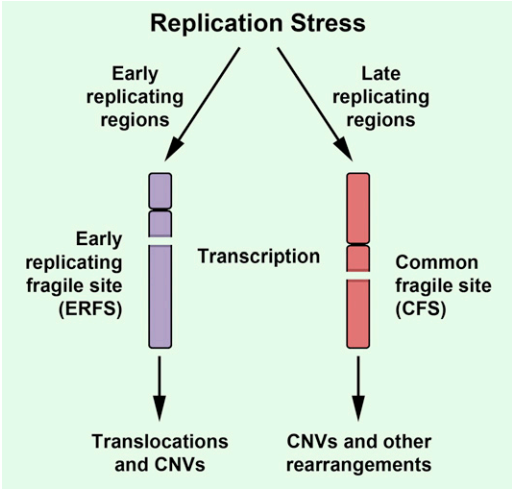

Figure 1. Comparison of Replication Stress-Induced Chromosome Breaks at ERFSs and CFSs
The difference in replication timing and the association with specific transcribed genomic regions are illustrated. ERFSs were found at regions that replicate early in S phase and are associated with early firing replication origins, whereas CFS regions replicate late and can be associated with intragenic, inactive origins and/or poor firing of dormant origins.

These studies have a number of implications. First, they identify a new class of fragile sites that are similar to CFSs in terms of chromosome breaks, sensitivity to replication stress, and dependence on ATR signaling. A notable difference is that ERFSs are associated with early replication origins, often in promoters, whereas CFSs replicate late, and at least some are associated with poor firing of late and dormant origins within large genes. A model thus emerges in which impaired replication is a universal contributor to breakage and associated rearrangements with the timing of replication stress leading to different, cell type-specific outcomes (Figure 1).

Second, the association of ERFSs with gene transcription is of great interest. A similar association has been made for some CFSs and transcription of large genes (Helmrich et al., 2011). These correlations are consistent with recent findings (Dellino et al., 2013) that suggest two classes of replication origins: those associated with moderate to high transcription levels and firing in early S and those associated with low transcription levels and firing throughout S. These and other studies raise important questions about the mechanistic connections between replication, origin firing, and transcription and the need to identify the genetic factors and epigenetic modifications involved. They also highlight the need for a thorough evaluation of genomic lesions in different cancer types based on differential transcription of the regions involved.

Finally, the association among ERFSs, translocations, and CNVs in B cell cancers is compelling. The data suggest that ERFSs can provide AID-independent DSBs that can partner with AID-induced or other DSBs to promote translocations, highlighting the importance of understanding the synergy between multiple simultaneous intrinsic and exogenous

genotoxic factors. In addition, the strong correlation of ERFSs with CNVs in B cell lymphomas strengthens the argument that common mechanisms likely underlie what might initially appear as distinct phenomena and suggest that replication arrest at ERFSs can trigger CNV formation. This is indeed likely, because similar CNVs that mimic constitutional and cancer-related CNVs are induced in human and mouse cells following replication stress, including at some CFSs (Arlt et al., 2011). It would be interesting to similarly examine de novo CNVs in B-lymphocytes treated to express ERFSs. The identity of the replication and repair factors that create the rearrangements and the endogenous conditions or environmental agents that lead to replication stress are unclear. These are important questions that are being addressed with regard to human germline CNVs and translocations, and they are equally important to understanding rearrangements in the cancer genome and the risk factors involved.

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ABT-199: Taking Dead Aim at BCL-2

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ABT-199 is a new selective small molecule inhibitor of BCL-2 that appears to spare platelets while achieving potent antitumor activity. Assays that can predict the efficacy of ABT-199 in individual tumors will be critical in determining how best to incorporate this promising agent into the armamentarium of cancer therapies.

The B cell lymphoma/leukemia 2 (BCL-2) family regulates critical life or death decisions of cells via the mitochondrial pathway of apoptosis (Davids and Letai, 2012). BCL-2 inhibits death by binding the BH3 domains of pro-death BCL-2 family proteins, thus preventing mitochondrial outer membrane permeabilization, which can be considered the point of commitment to apoptosis. BCL-2 has several anti-apoptotic cousins, including BCL-XL, BCL-w, and MCL-1, each of which possesses a distinct, hydrophobic BH3-binding pocket. Lymphoid malignancies are frequently addicted to BCL-2 for their survival. Because most of these cancers, including chronic lymphocytic leukemia (CLL), remain incurable with conventional therapies, agents that specifically target BCL-2 are under urgent investigation.

Early efforts to target the BCL-2 family were met with disappointment in the clinic. Agents such as the antisense oligonucleotide oblimersen sodium and the small molecule obatoclax showed promise as BCL-2 antagonists in preclinical testing but had little clinical activity. A potential mechanistic shortcoming of these agents is that they were never conclusively shown to specifically engage their purported BCL-2 family targets in patients.

Abbott Laboratories (now AbbVie) has developed a series of BH3-mimetic small molecules that bind to the BH3 binding sites of anti-apoptotic proteins like BCL-2. ABT-737, which binds BCL-2, BCL-XL, and BCL-w, was the first molecule studied extensively preclinically (Oltersdorf et al., 2005). Many subsequent experiments support its killing in an on-target fashion in cell lines, primary human cancer cells, and animal models. ABT-263 (navitoclax) was the first of this series to enter the clinic. Like ABT-737, it binds BCL-2, BCL-XL, and BCL-w, but it has the perceived advantage of being orally bioavailable. Clinical activity was observed, particularly in lymphoid cancers (Roberts et al., 2012); however, because navitoclax binds not only to BCL-2 but also to BCL-XL, the drug causes predictable,