Late Breaking Chromosomes

What a Difference an *ERCC1* or *ERCC4* (XPF) Mutation Makes!

Malignant disease is one of the major causes of mortality and imposes a significant burden on healthcare systems worldwide. The risk for incurring malignant disease for any given individual is difficult to predict. Yet, more than 50 genes involved in disorders, such as Xeroderma pigmentosum (XP), Fanconi anemia (FA), Ataxia telangiectasia, Nijmegen breakage syndrome, and Cockayne syndrome (CS) have been discovered. Although each of these syndromes exhibit distinct and recognizable patterns of clinical features, they all share genomic instability resulting from defective DNA damage responses. For instance, the 15 genes associated with FA [*FANCA*, *FANCB*, *FANCC*, *FANCD1* (*BRCA2*), *FANCD2*, *FANCE*, *FANCF*, *FANCG* (*XRCC9*), *FANCI*, *FANCL* (*BRIP1*), *FANCL* (*PHF9*), *FANCM*, *FANCN* (*PALB2*), *FANCO* (*RAD51C*), and *FANCP* (*SLX4*)] all participate in genome maintenance by protecting dividing cells against replication-blocking DNA lesions, such as interstrand crosslinks (ICL). CS results from defects in a transcription-coupled nucleotide excision repair pathway that removes DNA lesions caused by exposure to UV light [de Boer and Hoeijmakers, 2000]. Causative mutations for CS have been found in *ERCC8* (CSA) and *ERCC6* (CSB). In patients with a combined XP and CS phenotype, all identified mutations are in 3 of the XP-associated genes, *ERCC3* (XPB), *ERCC2* (XPD), and *ERCC5* (XPG). Notwithstanding this large number of candidate genes, an appreciable number of patients do not carry a mutation in any of the known genes.

Bogliolo et al. [2013] used whole-exome and Sanger sequencing on DNA of a FA patient who did not fit into any of the known complementation groups. Among the single nucleotide variants (SNVs), predicted by routine bioinfor-

matic procedures to be possibly pathogenic (such as *MUC17*), they discovered biallelic germline mutations in *ERCC4*. This gene encodes a structure-specific nuclease-encoding gene previously connected to XP complementation group F (XPF) and the segmental progeroid syndrome XFE, which makes it a possible candidate gene for this patient. Wild-type *ERCC4* cDNA complemented the phenotype of the patient's cell lines, thus, providing functional genetic evidence that mutations in *ERCC4* cause this FA subtype. Extensive biochemical and functional analyses demonstrated that the identified FA-causing *ERCC4* mutations disrupt XPF function in DNA ICL repair. Yet, the function of XPF in nucleotide excision repair was not significantly compromised. These results demonstrate that depending on the type of *ERCC4* mutation, either ICL or nucleotide excision repair can be compromised. As a result of the perturbed balance between both DNA repair activities, individuals present with one of the 3 clinically distinct disorders: FA, XP or XFE. Thus, the XPF endonuclease appears to be involved in several pathways of genome maintenance relating to distinct clinical disorders.

In an independent study, Kashiya et al. [2013] describe 3 CS individuals, who had no mutations in any of the known CS genes, but were deficient in *ERCC1* or *ERCC4* (XPF). A patient with phenotypes fitting XPF also had clinical features of CS and FA. Complementation fibroblast cultures from these patients with wild-type cDNA of *ERCC4* (XPF) or *ERCC1* rescued their defects in unscheduled DNA synthesis after exposure to UV light and mitomycin C. One patient carried a homozygous c.693C>G SNV in *ERCC1* exon 7, which alters amino acid Phe231 in the HhH domain of the protein. The other 2 patients share a heterozygous c.706T>C SNV in exon 4 of *ERCC4* and carry distinct SNVs in exon 8, which alter amino acids Cys236 and Tyr577, both in the SF2-like domain of the

ERCC4 gene. Expression studies confirmed the functional impact of the SNVs. Mice made homozygous for a frameshift mutation in *Ercc4* (Xpf) die by 3 weeks of age. Likewise, mice in which *Ercc1* has been deleted die soon after birth. These animals developed progressive neurodegeneration, leucopenia and thrombocytopenia, and impaired liver function. While these features resemble those of patients with XPCS1CD, pancytopenia is a hallmark of FA. These results, together with those from Bogliolo et al. [2013], underscore a multifunctional role for XPF and indicate that *ERCC4* and *ERCC1*, if defective, may be involved in either CS or the combined XP-CS-FA phenotype.

These studies have several ramifications. First, they demonstrate that depending on the type of *ERCC4* mutation the balance between NER and ICL-repair pathways may become altered, leading to one of 3 clinically distinct disorders in affected individuals. This resembles the case of XPD, which is either involved in XP complementation group D, Trichothiodystrophy (MIM 601675), or CS (MIM 216400), depending on the type of mutation [Broughton et al., 1995].

Second they highlight the pivotal role of XPF in preventing genome instability, malignant disease, bone marrow failure, developmental abnormalities, and premature aging.

Third, other human genetic disorders with an increased risk of malignant disease need to be reinvestigated. For instance, patients with the segmental progeroid

disorder, Werner Syndrome (WRN), have an increased risk for malignancy, a unique type of genomic instability, i.e. variegated translocation mosaicism, defective response to DNA damage, including ICLs, and lowered rates of homologous recombination [Salk et al., 1981a, b; Martin et al., 1999, Poot et al., 2001, 2002; Dhillon et al., 2007; Lauper et al., 2013].

Fourth, although the frequency of individual mutations in a single *FANC*, *XP*, *ERCC* gene is low [Kleijer et al., 2008], the aggregate number of individuals who carry a mutation in any of these genes becomes appreciable. The demonstrated cross talk between different pathways of DNA damage response opens the spectrum of possible digenic-triallelic mechanisms involving genes associated with different syndromes. Given that heterozygous carriers of *WRN* mutations exhibit elevated sensitivity to DNA-damaging agents [Ogburn et al., 1997; Poot et al., 1999], heterozygosity for mutated alleles of any of the *FANC*, *XP*, *CS* genes etc. may likewise affect DNA damage responses. Thus, the number of individuals in the general population at risk for malignant disease due to weakened DNA damage response may currently be underestimated. The novel genome-wide mutation screening techniques may give us a better insight in the near future and a point-of-entry into a more comprehensive genetic assessment of at-risk individuals in the general population.

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References

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