

## A Critical Role for Fanconi Anemia Proteins FANCG and FANCD2 in Centrosome Stability and Function

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Fanconi anemia proteins are involved in genome stability as they participate in the resolution of stalled replication forks via ATR-H2AX signalling (Bogliolo, Lyakhovich *et al.*, 2007, *EMBO J*; Lyakhovich and Surralles, 2007, *Cell Cycle*). However, not all genetic subtypes present the same clinical phenotype and evolution. Proteins downstream to the FA core complex seem to play additional roles as FANCD2 and FANCD1 patients have a more severe phenotype than patients with mutations in upstream genes. In addition, all FANCD2 patients always carry at least one hypomorphic mutation and the majority (89%) suffer from microcephalia (Kalb *et al.*, 2007, *Am J Hum Genet*) whereas FANCA patients tolerate biallelic N-ter truncating mutations or very large deletions (see abstract by Castella) and many have complete lack of FANCA with relatively mild phenotype (Callen *et al.*, 2005, *Blood*). FANCD2 and FANCD1 KO mouse models, but not FA core complex murine models, are cancer prone and FANCD1 KO hypomorphic mice (null mutations are embryo lethal), unlike FA core complex KO mice, are the only FA KO mice with an haematological syndrome *in vivo* (Navarro *et al.*, 2007, *Mol. Ther*). All these observations suggest alternative and independent roles for some of the FA proteins. Using proteomic analysis and biochemical fractioning, we found FANCD2 and FANCG at centrosomes. We also detected by mass spectrometry  $\gamma$ -tubulin interacting protein 2 in FANCD2-IP pellets resolved in 2D-PAGE. *In vivo* experiments with CFP-tagged FANCG and FANCD2 immunostaining demonstrate that FANCG and FANCD2 are present at centrosomes. Analysis of primary FA cells and retrovirally corrected wild type counterparts demonstrate elevated levels of centrosomal instability in FANCG and, to a lesser extent, FANCD2 deficient cells. Centrosome stability is restored in FANCG or FANCD2 deficient cells complemented with the corresponding wild-type cDNAs. Since centrosome stability is crucial for chromosome segregation, we also found elevated levels of chromosomal numerical abnormalities by dual colour FISH in FANCG and, marginally, FANCD2 deficient primary cells, phenotype that is again reverted in genetically complemented cells. Thus FANCG/FANCD2 are centrosomal proteins that play an essential role in centrosome stability and function. This role is independent of DNA repair, FANCD2 monoubiquitination and stalled replication forks and is probably coordinated with FANCD1/BRCA2, a FANCG-dependent but FANCD2 monoubiquitination-independent FANCD2 interacting protein with a known role in centrosome function. This leads us to suggest a novel centrosomal FA complex containing FANCG, FANCD1 and FANCD2. Given that centrosomal instability is a hallmark of cancer cells, we propose that cancer predisposition in FA could result from the co-occurrence of DNA repair deficiency and centrosomal dysfunction.