Modelling the Fanconi anemia/BRCA pathway and functional analysis of genetic variants by TALEN and CRISPR-Cas9

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Fanconi anemia (FA) is a rare genome instability disorder clinically characterized by developmental abnormalities, high predisposition to cancer and bone marrow failure. Many of the available cell lines from FA anemia patients are difficult to grow, difficult to transduce and genetically manipulate and are not isogenic. Due to current limitations of FA cell models, we decided to knock-out three FA genes (*FANCA, FANCD1, FANCQ*) and one associated gene (*FAN1*), all of them implicated in the FA/BRCA pathway, in the genetically amenable and fast growing human HEK293T cell line. We used transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats with CRISPR associated 9 nuclease (CRISPR-Cas9) for gene KO. Specific gene disruption was proven by genetic complementation with the corresponding wildtype gene using lentiviral vectors or transient transfection. Variants of uncertain significance (VUS) were introduced in the corresponding cDNA and functionally studied by lentivirus mediated genetic complementation. We successfully generated *FANCA, FAN1, FANCD1* and *FANCQ* KO HEK293T cell lines by the use of engineered nucleases. All these cell lines are sensitive to DNA crosslinking agents, thus mimicking cells from patients with defects in the FA/BRCA pathway. Moreover, all FA genes KO cells get blocked in the G2/M phase of the cell cycle upon treatment with DNA crosslinkers, a typical FA cellular phenotype. Finally, these FA cell lines reproduce gene-specific defects: *FANCA-/-* cells have impaired FANCD2 monoubiquitination, *FANCD1-/-* cells are unable to form Rad51 foci after irradiation and are sensitive to PARP inhibitors, and *FANCQ -/-* cells are sensitive to UV-light radiation and were amenable for functional analysis of VUS.