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Mini review

FANCJ: Solving problems in DNA replication

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ABSTRACT

The FANCJ protein (also known as BACH1 and BRIP1) is a DNA helicase that is required to preserve the genetic and structural integrity of the genome in complex eukaryotes. In humans, mutations in FANCJ are associated with the chromosome instability disorder Fanconi's anemia and also with the inherited predisposition early-onset breast cancer. Here I will discuss the contribution of FANCJ to human disease, its role in maintenance of genome stability and some current thoughts on the mechanisms through which this is achieved.

Contents

The FANCJ protein (also known as BACH1 and BRIP1) is a DNA helicase that is required to preserve the genetic and structural integrity of the genome in complex eukaryotes. In humans, mutations in FANCJ are associated with the chromosome instability disorder Fanconi's anemia [1–3] and also with the inherited predisposition early-onset breast cancer [4,5]. Here I will discuss the contribution of FANCJ to human disease, its role in maintenance of genome stability and some current thoughts on the mechanisms through which this is achieved.

1. FANCJ and cancer

FANCJ was first identified as a protein that binds directly to the breast cancer associated tumour suppressor BRCA1 [5]. It was originally named BACH1 (BRCA1-Associated C-terminal Helicase1), but was subsequently renamed BRIP1 (BRCA1 Interacting Protein C-terminal Helicase1) to avoid confusion with a transcription factor of the same name. More recently, its identification as a component of the Fanconi anemia pathway (see below), has led to it being more commonly referred to as FANCJ. For the sake of clarity I will refer to this protein simply as FANCJ.

Its association with BRCA1 raised the possibility that defects in FANCJ might, like those in BRCA1, be linked with the inherited predisposition to early-onset breast cancer. This notion was supported by the identification of two different mutations, which alter helicase function of FANCJ, in the germlines of two patients with early-onset breast cancer but not in matched controls [5]. Subsequently, a larger genetic study of breast cancer patients that do not have mutations in either BRCA1 or BRCA2, identified inactivating truncation mutations in FANCJ. This supported the hypothesis that monoallelic carriers of these mutations may confer increased susceptibility to breast cancer [4].

The tumour suppressor function of BRCA1 is thought to derive from its pivotal role in the DNA damage response and, more

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specifically, in the accurate repair of DNA double stranded breaks (DSB) by homologous recombination (HR) [6]. Several pieces of evidence suggest that FANCJ might also contribute to this function. Firstly, the interaction of FANCJ with the BRCT domains of BRCA1 is dependent on its prior phosphorylation during S-G2 phase of the cell-cycle when HR is most active [7]. Secondly, the interaction of FANCJ with BRCA1 coincides with the dynamic re-localization of these two proteins in discreet nuclear foci at sites of DNA damage/on-going replication [8]. Thirdly, BRCA1 and FANCJ are components of a much larger complex containing other proteins associated with the DNA damage response, such as TopBP1 and MutLα (comprising MLH1 and PMS2) [9,10]. Finally, the expression of a helicase-dead form of FANCJ in cells results in the accumulation of unrepaired DNA breaks [5].

The contribution of FANCJ to the repair of a restriction endonuclease-generated DSB has been tested directly in cells. Using this assay Litman et al. [3] reported that knockdown of FANCJ in human cells with small interfering RNA (siRNA), resulted in a 10-fold reduction in the repair of DSBs by HR, which compares with the 5–10-fold defect in HR-mediated DSB repair reported for cells that are defective in BRCA1 [6]. However, this role in HR does not appear to be conserved through evolution. Similar studies using *fancj* mutant cells derived from the avian cell line DT40, showed no defect in HR-mediated repair of an I-Sce-1 induced DNA break [11]. Likewise in *C. elegans*, mutation of the FANCJ homolog Dog-1 does not appear to adversely affect HR [12].

Another important function of BRCA1 in the preservation of genomic integrity is its role in the execution of a G2/M cell-cycle checkpoint. Yu et al. reported that cells defective for either BRCA1 or FANCJ exhibit similar checkpoint defects, each failing to arrest at G2/M phase after treatment with ionizing radiation [13]. Moreover, this checkpoint function depends on the phosphorylation of FANCJ on serine 990, which is required for its interaction with the BRCT domains of BRCA1. It is possible, however, that this function is limited to specific types of DNA damage because cells from FA-J patients and DT40-derived *fancj* mutant cells both exhibit a robust checkpoint at G2-M in response to agents such as cisplatin and mitomycin C, which cause interstrand DNA crosslinks [11,14].

The interaction of FANCJ with BRCA1 has also been implicated in the timely progression of cells through S-phase by assisting in the resolution of stalled replication forks. Kumaraswamy and Shiekhattar proposed that this function is dependent on the assembly of a complex containing BRCA1, BRCA2 and FANCJ as cells enter S-phase and correlates with an increase in the DNA-dependent ATPase activity of FANCJ and its localization to chromatin [15]. By contrast, in G1 the FANCJ helicase is silenced through the dephosphorylation of this complex at an as yet unidentified site.

The association of FANCJ with BRCA1 and its potential link with predisposition to breast cancer is both fascinating and potentially important. However, two pieces of evidence suggest that this relationship is not straightforward. Firstly, mutations in BRCA1 and FANCJ have markedly different penetrance with regard to early-onset breast cancer, the former being extremely high and the latter very low [4]. Secondly, the inheritance of biallelic mutations in FANCJ, which causes another human genome instability disease called Fanconi's anemia (discussed below), causes in increased susceptibility to cancers of the haemopoeitic system, such as AML, and to tumours of the head and neck, but not to breast and ovarian cancer.

It seems likely, therefore, that only a small part of the tumour suppressor function of BRCA1 is contributed through a common pathway with FANCJ. The discovery that FANCJ is associated with another human genome instability disease, Fanconi anemia, has provided a more tangible explanation for the role of FANCJ in the DNA damage response

2. FANCJ and Fanconi anemia

Fanconi anemia (FA) is an inherited chromosome instability disease, which defines a genetic pathway for the repair of replication blocking lesions such as interstrand DNA crosslinks (ICL). Patients with this disease commonly suffer developmental abnormalities and are characterized by progressive bone marrow failure during the first decade of life [16]. FA is also associated with increased susceptibility cancers such as acute myeloid leukaemia and tumours of the head and neck [17]. An important diagnostic indicator is the marked sensitivity of FA cells to DNA crosslinking agents such as mitomycin C and an associated increase in chromosomal aberrations, such as chromatid breaks and interchanges features, which are characteristic of a defect in the repair of DNA damage [18,19].

FA is genetically complex [20]. Work over several decades has identified 13 genes, which are defective in patients with an FA phenotype. There are also a number cell lines from FA patients for which the gene defect is yet to be discovered and a plethora of factors that are functionally associated with the FA pathway, but are not represented in patients. Of the known FA proteins, very few have yielded clues as to their molecular function and consequently the mechanism through which the FA pathway operates is still largely unclear. Nevertheless, biochemical activities have been demonstrated for some FA proteins, including FANCJ, and these have provided a few clues to the mechanism of FA-mediated DNA repair in cells.

Currently, the defining molecular event associated with the FA pathway relates more closely with the transduction of a signal that activates the pathway, than to a mechanism by which DNA lesions are repaired. This signal involves the ubiquitylation of a key component in the FA pathway, comprising a complex of the FANCD2 and FANCI proteins (D2/I) [21,22]. Ubiquitylation of D2/I is prerequisite for the repair of DNA crosslink damage by the FA pathway and coincides with its dynamic relocalization to specific sites on

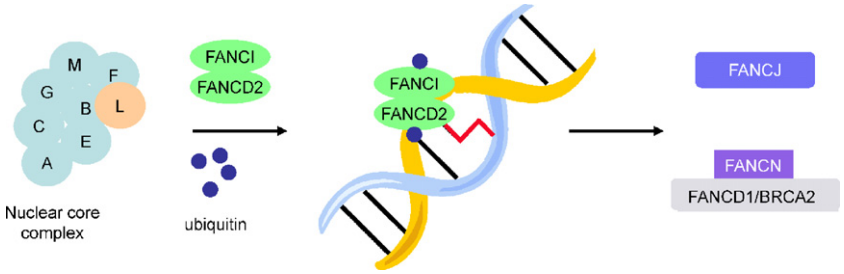

Fig. 1. Schematic representation of the FA pathway. Only proteins with defects identified in Fanconi Anemia patients are shown. Eight FA proteins form a large nuclear 'core' complex. Of these FANCM is a putative DNA translocase/nuclease and FANCL (depicted in yellow) is an ubiquitin ligase. Although FANCL interacts with the ubiquitin conjugating protein Ube2T (not shown) to facilitate ubiquitin transfer, all members of the nuclear core complex are required for the efficient ubiquitylation of FANCI/D2 complex. Ubiquitylation of FANCI/D2 correlates with its relocalization to specific sites on chromatin. FANCJ, FANCN and FANCD1 are not required for the ubiquitylation of FANCI/D2 and so are likely to function in parallel with, or downstream of the main FANCI/D2 associated FA pathway.