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Review

The underlying mechanism for the PARP and BRCA synthetic lethality: Clearing up the misunderstandings

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ABSTRACT

Poly (ADP-ribose) polymerase (PARP) inhibitors effectively kill tumours defective in the *BRCA1* or *BRCA2* genes through the concept of synthetic lethality. It is suggested that PARP inhibitors cause an increase in DNA single-strand breaks (SSBs), which are converted during replication to irreparable toxic DNA double-strand breaks (DSBs) in BRCA1/2 defective cells. There are a number of recent reports challenging this model. Here, alternative models that are not mutually exclusive are presented to explain the synthetic lethality between BRCA1/2 and PARP inhibitors. One such model proposes that PARP inhibition causes PARP-1 to be trapped onto DNA repair intermediates, especially during base excision repair. This may in turn cause obstruction to replication forks, which require BRCA-dependent homologous recombination to be resolved. In another model, PARP is directly involved in catalysing replication repair in a distinct pathway from homologous recombination. Experimental evidence supporting these novel models to explain the PARP-BRCA synthetic lethality are discussed.

1. Introduction

Inherited mutations in one copy of either the *BRCA1* or *BRCA2* gene is associated with a high risk of developing primarily breast and ovarian cancer (Miki et al., 1994; Wooster et al., 1995). Cancers arising in these individuals have lost a functional copy of *BRCA1* or *BRCA2*. Hence, the BRCA1 and BRCA2 proteins are tumour suppressors and are required for homologous recombination (HR) to suppress genetic instability, which can lead to cancer (Venkitaraman, 2002). BRCA1 and BRCA2 defective tumours are intrinsically sensitive to PARP inhibitors, both in tumour models *in vivo* (Bryant et al., 2005; Evers et al., 2010; Farmer et al., 2005; Liu et al., 2007; Rottenberg et al., 2008) and in the clinic (Fong et al., 2009). Only mild side effects have been reported from PARP inhibitor treatment (Fong et al., 2009), which can be attributed to PARP

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inhibitors selectively targeting BRCA defective cells, owing to their defect in HR (Bryant et al., 2005; Farmer et al., 2005). Normal cells, with intact HR, are not significantly affected, in line with evidence that PARP-1$^{-/-}$ mice are alive and healthy in general (de Murcia et al., 1997; Wang et al., 1997).

The genetic interaction between PARP and BRCA can be described as synthetic lethal. Synthetic lethality between two genes occurs where individual loss of either gene is compatible with life, but simultaneous loss of both genes results in cell death. It has for a long time been suggested that a synthetic lethal approach could be used in the treatment of cancer (Hartwell et al., 1997) and the PARP-BRCA interaction provides the first example of a successful synthetic lethal approach that has entered the clinic.

Although several years have passed since the initial reports on the PARP-BRCA synthetic lethality, we have so far not seen any other synthetic lethal approach reach the clinic. One possible reason for the slow pace in the development of new drugs using this concept may be our inability to mechanistically explain the PARP-BRCA synthetic lethality. Indeed, mechanistic understanding has not been helped by the publication of numerous statements without support from the literature. Here, I will review recent findings that affect our mechanistic understanding of the PARP-BRCA synthetic lethality.

2. PARP-1 is not a base excision repair protein

It is well established that the PARP-1 protein binds to SSBs, where it is activated to convert NAD^+ into ADP-ribose polymers (PAR), and that the protein is required for efficient SSB repair (Fisher et al., 2007; Satoh and Lindahl, 1992; Strom et al., 2011) by attracting XRCC1 to the site of damage (El-Khamisy et al., 2003) (Figure 1A).

Traditionally, BER has been suggested to work as a series of independent steps, starting with removal of the damaged base, followed by separate recognition by AP-endonuclease (APE), which makes a SSB incision. This unprotected SSB acts as a substrate for SSB repair (SSBR) involving PARP-1 (Figure 1B). Indeed, PARP-1 has been suggested to have a role in BER (Dantzer et al., 1999, 2000). This suggestion is well founded, as PARP-inhibited or PARP-1$^{-/-}$ cells are hypersensitive to agents that cause base lesions (de Murcia et al., 1997; Wang et al., 1997) and PARP-1 is required for the rapid closure of alkylation-induced SSBs (Trucco et al., 1998). Furthermore, ligation of AP-sites generated from uracil or 8-oxoguanine lesions is delayed in extracts from PARP-1$^{-/-}$ cells. A potential caveat of these experiments is that damaged DNA and AP-sites can be heat sensitive, which may cause these lesions to be converted into SSBs (Lundin et al., 2005). In addition, alkylated DNA bases effectively block replication elongation (Groth et al., 2010), and the sensitivity in PARP-1$^{-/-}$ cells to those agents may be related to a role for PARP-1 at replication forks (see below).

Other scientists have reported that BER kinetics are reduced in the presence of the active PARP-1 protein (Allinson et al., 2003). Thus, the role of PARP-1 in BER has remained elusive. Recently, we have set up an assay to measure BER incision in cells and the half-life of the SSB intermediate formed during BER (Strom et al., 2011). Using this assay, we find that PARP-1 is not required for BER in cells, but rather that the presence of PARP-1 protein reduces the BER turnover (Strom et al., 2011). These data support a model where BER occurs in a single, coordinated

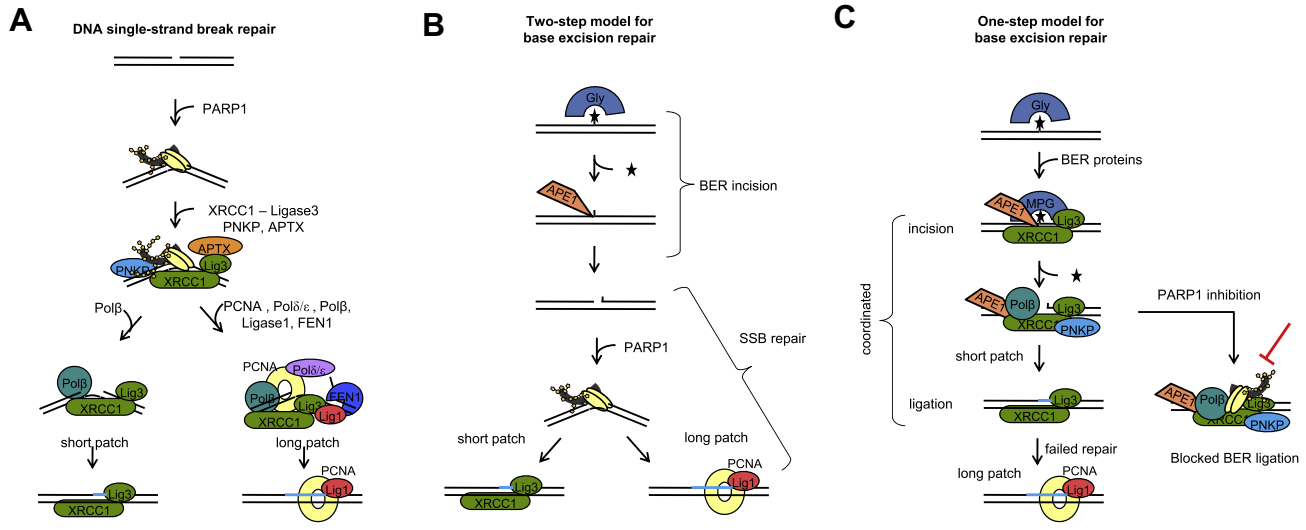

Figure 1 – Base excision repair (BER) is a separate process from DNA single-strand break (SSB) repair in mammalian cells, although the two processes share proteins. (A) *SSB repair*: PARP-1 has a high affinity for SSBs and will be amongst the first proteins to bind to the lesion. In turn PARP recruits factors to start end processing and finally ligation, normally through short patch repair and through long patch repair where the lesions are more difficult to repair. (B) *Two-step model for BER*: Different base lesions are recognised by different glycosylases (Gly), which are excised before SSB incision by the AP-endonuclease (APE). These SSBs are then left unprotected and recognised in a separate process by PARP-1 that will then initiate SSB repair. (C) *One-step model for BER*: The glycosylase interacts with proteins involved in the early BER incision step and excises the damaged base shortly before APE incision. The half-life of the SSB intermediate is very short and rapidly ligated by short patch repair, which switches to long patch repair in case of ligation difficulty. PARP-1 has no role in BER, but can transiently bind the SSB intermediate. When PARP-1 is inhibited, it can be trapped on the SSB intermediate and prevent the ligation step.