From the Department of Oncology-Pathology
Karolinska Institutet, Stockholm, Sweden

DNA REPAIR BY HDR IN EXPERIMENTAL TUMORIGENESIS

Ulrica Westermark

Karolinska Institutet

Stockholm 2008

Published by Karolinska Institutet and printed by Larserics Digital Print AB

ISBN 978-91-7409-002-4

To my family

ABSTRACT

The aim of this thesis was to further understand how defects in the homology-directed repair (HDR) pathway affect tumor formation and development. To study proteins involved in HDR is challenging since most of its members are required for cell viability. For our studies we have therefore taken a dominant-negative approach to address the importance of the BRCA1 interacting protein BARD1 in HDR (paper I) and investigate the role of HDR in PDGFB-induced gliomagenesis by interfering with a key protein, RAD51 (paper II). In addition we have also investigated how Platelet-derived growth factor A receptor (*PDGFRA*) is transcriptionally regulated by interleukin-1β (IL-1β) (paper III).

The breast cancer susceptibility gene BRCA1 is frequently mutated in hereditary breast and ovarian cancers. BRCA1 has been implicated in many different cellular processes, among them DNA repair by HDR. In Paper I we investigated if BARD1 was involved in HDR through its interaction with BRCA1. We could show that expression of a truncated BARD1 decreased HDR of an induced double-strand break and that this decrease was even more pronounced in Brca1-deficient cells expressing a splice variant of Brca1 that still are able to bind BARD1. We could also show that the role of BARD1 in HDR was dependent on binding to BRCA1 and that the HDR defect resulting from the truncated BARD1 was caused by lack of regulatory elements in the C-terminal end of BARD1 and not caused by improper cellular localization of either the BARD1 construct or the endogenous Brca1 protein. We conclude that BARD1 is important for HDR and that the repair function of BARD1 is dependent on its interaction with BRCA1.

The expression of PDGFRα is strictly regulated during embryogenesis and aberrant PDGFRα expression can lead to malignant transformation, in brain tumors for example. Highly malignant gliomas are the most frequent primary tumor of the central nervous system in adults. To study the effect of different genetic changes in gliomagenesis, glioma-like tumors can be induced by intracerebral injections of oncogene carrying retroviruses. The RCAS/tv-a model system provides the possibility to study combinations of different genetic alterations in a specific cell type. In paper II we have used wild type and *Arf-/-* nestin *tv-a* (*Ntv-a*) transgenic mice, where expression of the RCAS constructs were directed to neural progenitor cells. It has previously been shown that expression of PDGFB can induce glioma-like tumors in these mice. To investigate the role of HDR in tumorigenesis we co-expressed RAD51 or a DNA repair deficient RAD51 (RAD51KR) contruct with PDGFB in either wild type or *Arf-/- Ntv-a* mice. We could show that co-expression of RAD51 or RAD51KR can suppress PDGFB-induced tumorigenesis in wild type mice. However, only RAD51 was able to suppress tumor formation in the Arf-null background. We could also show that all the PDGFB-induced tumors were aneuploid, independent of genotype, tumor grade or tumor size. Interestingly, expression of RAD51 or RAD51KR reduced aneuploidy in the PDGFB-induced tumors.

LIST OF PUBLICATIONS

I. **Westermark UK,** Reyngold M, Olshen AB, Baer R, Jasin M, Moynahan ME. BARD1 participate with BRCA1 in homology-directed repair of chromosome breaks. *Mol. Cell. Biol. 2003;23(21):7926-7936*

II. **Westermark UK,** Forsberg N, Bråsäter D, Helgadottir HR, Eriksson A, Zetterberg A, Jasin M, Nistér M, Uhrbom L. RAD51 suppresses PDGFB-induced gliomagenesis and genetic instability in wild type and $Arf^{-/-}$ mice. *Submitted*

III. Afink G, **Westermark UK**, Lammerts E, Nistér M. C/EBP is an essential component of PDGFRA transcription in MG-63 cells. *BBRC 2004;315(2):313-318*

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ARF	$p14/p19^{Arf}$
ATM	Ataxia Telangiectasia Mutated
ATR	Ataxia Telangiectasia and Rad3 related
BARD1	BRCA1-associated RING domain 1
B-cell	Neural stem cell
BLM	Bloom syndrome gene
BRCA	Breast cancer
BRCT	BRCA1 COOH-terminal
CDK	Cyclin dependent kinase
C/EBP	CCAT/enhancer-binding element
CHO	Chinese hamster ovary
CNS	Central nervous system
DDR	DNA damage response
DR-GFP	Direct repeats-green fluorescent protein
DNA-PK	DNA-protein kinase
DSB	Double strand break
EGFR	Epidermal growth factor receptor
ES cell	Embryonic stem cell
FGF	Fibroblast growth factor
GBM	Glioblastoma multiforme
GFAP	Glial fibrillary acidic protein
γH2AX	Phosphorylated H2AX
HDR	Homology-directed repair
IL-1β	Interleukin-1β
INK4a	Inhibitors of kinase 4a, p16
LOH	Loss of heterozygosity
MEF	Mouse embryonic fibroblast
MMC	Mitomycin C
MMLV	Moloney murine leukemia virus
MRN	Mre11/RAD50/Nbs1
NHEJ	Non-homologous end joining
NLS	Nuclear localization signal

Ntv-a	Nestin tv-a
OIS	Oncogene induced stress
p53	Tp53 gene product
PDGF	Platelet derived growth factor
PDGFR	Platelet derived growth factor receptor
PI3K	Phosphatidylinositol 3-kinase
PIKK	Phophoinositide 3-kinase-related protein kinase
RCAS	Replication competent ALV splice acceptor
RPA	Replication protein A
RTK	Receptor tyrosine kinase
SVZ	Subventricular zone
Tv-a	Receptor for RCAS
UTR	Untranslated region
WRN	Werner syndrome gene

1 HOMOLOGY DIRECTED REPAIR

1.1 INTRODUCTION

Tumorigenesis is known to result from multiple genetic changes. To protect the cell against these changes rigorous cellular mechanisms exist to repair DNA damage caused by endogenous and environmental insults, or to eliminate those cells that are irreparably damaged. It is of great importance for the maintenance of genome integrity to repair DNA breaks. Failure to repair these lesions could lead to genomic instability, a common feature of nearly all solid tumors (Lengauer *et al.*, 1998). There are two major DNA repair pathways to repair double strand breaks (DSBs) in mammalian cells; non-homologous end joining (NHEJ) and homology directed repair (HDR) (also called homologous recombination). The NHEJ pathway is potentially error-prone, allowing deletion and insertion of nucleotides at the site of the DSB. HDR, on the other hand, is error-free, when an identical sister chromatid is used as template for the repair. The HDR pathway also play a pivotal role in replication, where it guards and repair replication errors at the replication forks (Pierce *et al.*, 2001). The HDR pathway will be the DNA repair pathway of primary focus in this thesis.

1.2 HDR PATHWAY

In response to DNA damage, checkpoint kinases are activated (e.g. ATM, ATR, DNA-PK). The DSB lesions are recognized by the Mre11/Rad50/ Nbs1 (MRN) complex (Carson *et al.*, 2003). The MRN complex recruits ATM to the site of the break by direct binding to ATM and Nbs1. ATM phosphorylates histone H2AX (γ-H2AX), which in turn works as a docking site for Mdc1. Mdc1 binds both γ-H2AX and Nbs1 and can facilitate spreading of H2AX phosphorylation, thereby promotes remodeling of the chromatin. Other proteins known to be recruited to sites of DSBs at this point are 53BP1 and the BRCA1-BARD1 complex. The accumulation of ATM phosphorylates ATM targets, such as the Chk2 kinase. This initial response at the chromatin is very fast and these proteins are recruited to the site of the break within a minute of the DSB formation (Bartek and Lukas, 2007). Of note, exactly how and in which order these proteins are recruited to DSBs is not completely understood and hence the above description represent one suggested scenario.

ATM and the MRN complex are also important for resection of DNA around the break creating a single stranded DNA (ssDNA) to facilitate ATR-dependent signaling and DNA repair by HDR. Resection of DNA is only possible in the S and G2-phase of the cell cycle and entails activation of cyclin-dependent kinases (CDKs) (Ira *et al.*, 2004). The ssDNA is stabilized and coated by replication protein A (RPA). It has recently been shown that RPA plays an active role in initiating HDR by binding RAD52 and recruiting RAD51 to the break (Sleeth *et al.*, 2007). This process is dependent on the checkpoint kinase Chk1. Chk1 is needed for dissociation of RPA from ssDNA and subsequent loading of RAD51 onto the ssDNA. It was also shown that in cells lacking RPA DSBs persists (Sleeth *et al.*, 2007).

The central protein to repair DSBs by HDR is the RecA homolog RAD51. RAD51 is loaded onto the ssDNA, a process aided by BRCA2 (Yang *et al.*, 2005) and Chk1 (Sleeth *et al.*, 2007), replacing RPA. RAD51 forms nucleoprotein filaments in an ATP-dependent manner around the ssDNA. This is a central event in HDR since it catalyses DNA strand exchange reaction between ssDNA and the homologous double-stranded DNA (dsDNA) in the sister chromatid (Baumann *et al.*, 1996; Benson *et al.*, 1994; Gupta *et al.*, 1997). The accumulated RAD51 can be visualized as so called nuclear foci (Haaf *et al.*, 1995; Tan *et al.*, 1999). In these foci RAD51 co-localizes with the RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3) (Masson *et al.*, 2001), other members of the RAD52 epistasis group (e.g. RAD52, RAD54) (Golub *et al.*, 1997; Tan *et al.*, 1999) as well as other proteins implicated in DNA repair, such as the breast cancer susceptibility genes BRCA1 and BRCA2 (Breast cancer 1 and 2) (Chen *et al.*, 1998; Scully *et al.*, 1997; Sharan *et al.*, 1997), BARD1 (Brca1 associated Ring domain 1) (Jin *et al.*, 1997; Wu *et al.*, 1996), γH2AX (Paull *et al.*, 2000), 53BP1 (Schultz *et al.*, 2000) and FANCD2 (Fanconi anaemia D2) (Garcia-Higuera *et al.*, 2001) amongst other known and unknown proteins.

Nearly all proteins involved in the HDR pathway are essential for cell survival, as most of the HDR genes cause embryonic lethality when knocked-out in mouse models (e.g. *Rad51, Brca1, Bard1, Brca2, Rad51B, Rad51C, Rad51D and xrcc2*) (Deans *et al.*, 2000; Gowen *et al.*, 1996; Lim and Hasty, 1996; Liu *et al.*, 1996; McCarthy *et al.*, 2003; Pittman and Schimenti, 2000; Smiraldo *et al.*, 2005). Some of them can be rescued or embryonic life can be extended by p53 deficiency (Hakem *et al.*, 1997; Ludwig *et al.*, 1997; McCarthy *et al.*, 2003; Smiraldo *et al.*, 2005). Cells with defects in

the HDR pathway show extensive chromosomal instability, sensitivity to cross-linking agents and irradiation (Jeggo, 1998; Moynahan *et al.*, 2001a; Moynahan *et al.*, 2001b; Sonoda *et al.*, 1998; Thompson and Schild, 1999). This implies that disruption in recombination processes has devastating consequences to genome integrity and that this could potentially lead to tumorigenesis. On the other hand, the sensitivity of HDR mutants to DNA damaging agents has also been suggested to be potential targets for cancer therapy.

1.3 RAD51

RAD51 is cell cycle regulated with highest expression in S/G2-phase of the cell cycle and lowest in resting cells. In S-phase RAD51 can be found in discrete nuclear foci located at replication forks (Flygare *et al.*, 1996; Haaf *et al.*, 1995; Scully *et al.*, 1997). Upon DNA damage RAD51 is relocated to sites of DSBs (Haaf *et al.*, 1995; Raderschall *et al.*, 1999).

Exactly how RAD51 is regulated is not completely understood. However, it has been shown that p53 can regulate RAD51 by binding to the oligomerization domain of RAD51 and thereby preventing nucleoprotein filament formation (Linke *et al.*, 2003; Sturzbecher *et al.*, 1996). BRCA2 can also regulate RAD51 either by interaction via its BRC repeats, thereby keeping RAD51 in an inactive monomeric state or stabilizing RAD51 by binding the RAD51 polymer through the C-terminal end of BRCA2 (Davies and Pellegrini, 2007; Esashi *et al.*, 2005; Esashi *et al.*, 2007; Galkin *et al.*, 2005; Petalcorin *et al.*, 2007). It has also been shown that RAD51 can be phosphorylated and upregulated by the STAT5 pathway (Slupianek *et al.*, 2002; Slupianek *et al.*, 2001).

Disruption of *Rad51* in mouse embryonic tissue leads to lethality early in gestation (Lim and Hasty, 1996; Tsuzuki *et al.*, 1996), and the inducible loss of the Rad51 protein in chicken DT-40 cells resulted in severe chromosome aberrations and cell death (Sonoda *et al.*, 1998). Overexpression of RAD51, on the other hand, has also been reported to lead to genomic rearrangements (Richardson *et al.*, 2004). It has also been shown that elevated RAD51 levels can increase HDR, which could possibly lead to DNA repair with less fidelity (Lundin *et al.*, 2003; Richardson *et al.*, 2004; Vispe *et al.*, 1998; Xia *et al.*, 1997). This implicates the importance of proper regulation of

RAD51 in maintaining genome integrity, which is believed to be of utmost importance to prevent malignant cell transformation.

1.3.1 Rad51 in tumorigenesis

Mutations in RAD51 are rarely found in human tumors (Kato *et al.*, 2000; Maacke *et al.*, 2000a; Maacke *et al.*, 2000b), but deletions of the chromosomal region where RAD51 is located (15q15.1) and subsequent loss of heterozygozity (LOH) in breast cancer has been reported (Gonzalez *et al.*, 1999; Nowacka-Zawisza *et al.*, 2007). Moreover, there are several reports showing that a polymorphism in the 5' untranslated region (UTR) of RAD51 increases the risk of breast cancer in carriers of mutations in the breast cancer associated gene BRCA2 (Antoniou *et al.*, 2007; Jara *et al.*, 2007; Kadouri *et al.*, 2004; Levy-Lahad *et al.*, 2001). It has been suggested that this polymorphism leads to a decrease in RAD51 levels (Antoniou *et al.*, 2007). RAD51 also interacts with proteins known to be involved in tumorigenesis, such as p53 (Sturzbecher *et al.*, 1996), BLM (Wu *et al.*, 2001), BRCA1 (Scully *et al.*, 1997) and BRCA2 (Sharan *et al.*, 1997), suggesting a direct or indirect role for RAD51 in tumorigenesis.

Overexpression of RAD51 is found in several different types of tumor tissues as well as in tumor derived cell lines (Han *et al.*, 2002; Maacke *et al.*, 2000a; Maacke *et al.*, 2000b; Raderschall *et al.*, 2002b; Xia *et al.*, 1997; Yanagisawa *et al.*, 1998). This overexpression seems not to be caused by amplification of the RAD51 gene (Raderschall *et al.*, 2002b), suggesting that the overexpression is due to increased transcription or post-translational modifications. Secondary effects of mutations in regulators of RAD51 could also cause the elevated levels of RAD51 seen in tumor tissues. TP53, for example, has been suggested to inhibit RAD51 expression through direct interaction and as a consequence of that RAD51 expression has shown to be elevated in p53 deficient cells (Arias-Lopez *et al.*, 2006; Hannay *et al.*, 2007). Similarly, overexpression of RAD51 could reflect the disruption of its normal cleavage by caspase-3 during apoptosis (Huang *et al.*, 1999), since the apoptotic pathways commonly are bypassed in malignant cells. For example, tumor cells expressing different fusion tyrosine kinases (e.g. BCR/ABL, TEL/PDGFRβ, TEL/ABL, TEL/JAK2) have elevated levels of RAD51 and they show increased resistance to DNA damaging drugs. These fusion tyrosine kinases upregulate STAT5 expression

(Slupianek *et al.*, 2002) and STAT5 has been shown to transactivate the RAD51 promoter and inhibit RAD51 cleavage by caspase-3 (Slupianek *et al.*, 2001). Hence, RAD51 is upregulated in these cells and contributes to drug resistance (Slupianek *et al.*, 2002). The replicative stress caused by upregulation of oncogenes in tumor tissue and the DNA breaks caused by that could potentially also lead to elevated levels of RAD51 in tumor tissue.

Overexpression of RAD51 has also been correlated to histological grade in breast carcinomas (Maacke *et al.*, 2000b). In this study no correlation was found between RAD51 overexpression and BRCA1 deficiency. However, BRCA1 deficiency had an inverse correlation to tumor grade (Maacke *et al.*, 2000b). Other studies have found that RAD51 was upregulated in BRCA1-deficient tumors (Honrado *et al.*, 2005; Martin *et al.*, 2007) at a higher frequency than in sporadic tumors and that this higher RAD51 expression was independent of cell cycle variables (Martin *et al.*, 2007). In the same study by Martin *et al.* it was further shown that growth and DNA repair defects in BRCA1-deficient chicken DT-40 cells could be rescued by upregulation of the HDR pathway. They suggested that elevated HDR activity in BRCA1-deficient cells could provide a permissive genetic context for tumor development in carriers of BRCA1 mutations (Martin *et al.*, 2007). In normal cells BRCA1 interacts and inhibits c-Abl. Loss of BRCA1 leads to constitutively elevated c-Abl levels (Foray *et al.*, 2002). In turn, RAD51 can be phosphorylated and upregulated by c-Abl (Chen *et al.*, 1999; Yuan *et al.*, 1998) through the STAT5 pathway, as mentioned above. This could be one explanation why RAD51 is upregulated in a BRCA1-deficient background.

1.3.2 RAD51 overexpression in experimental models

Overexpression of RAD51 *in vitro* has shown to decrease proliferation, delay cell cycle progression, increase apoptosis and increase resistance to DNA damaging treatments (Flygare *et al.*, 2001; Ohnishi *et al.*, 1998; Raderschall *et al.*, 2002a; Russell *et al.*, 2003; Vispe *et al.*, 1998). High levels of RAD51 can lead to higher order nuclear structures in experimental models (Raderschall *et al.*, 2002a; Westermark *et al.*, 2008). Wheather these structures also occur in human tumors overexpressing RAD51 is unclear and the importance of these structures in tumorigenesis is so far unknown.

The role of overexpression of RAD51 in maintaining genome integrity remains elusive. One report shows that overexpression of RAD51 in a variety of different human cell lines led to fewer chromosomal aberrations (Raderschall *et al.*, 2002a). In paper II in this thesis, we show that RAD51 overexpression has a protective effect on genome stability, in a mouse tumor model (Westermark *et al.*, 2008). However, it has also been shown in a mouse embryonic stem (ES) cell model that overexpression of RAD51 can lead to increased chromosomal instability (Richardson *et al.*, 2004). However, it was later shown that overexpression of RAD51 in ES cells generated more translocations after an induced DSB compared to myeloid cells (Francis and Richardson, 2007), suggesting cell specific differences in the response to RAD51 overexpression.

A study by Lundin *et al.* has provided a possible explanation to the observed decrease in genomic instability by RAD51 overexpression (Lundin *et al.*, 2003). They induced different types of DNA lesions at replication forks in cells overexpressing RAD51, using etoposide (VP16) and hydroxy urea (HU) to induce DSBs at the replication forks and thymidine to slow down replication without inducing DSB. However, thymidine treatment leads to assembly of HDR proteins at replication forks, implying that thymidine induces a DNA damage response before DSBs are formed. They could show that RAD51 was involved in repairing all these different types of lesions. They could also show that cells overexpressing RAD51 more efficiently repaired VP16 induced DSB and that overexpression of RAD51 suppressed long-tract HDR but not global HDR. They propose that RAD51 can rescue stalled replication forks before DSB formation, supported by the involvment of RAD51 in thymidine lesions. This data taken together implies that overexpression of RAD51 could protect against genomic instability by more efficient DSBs repair, rescuing stalled replication forks and thereby preventing formation of DSBs. Suppression of long-tract HDR could also benefit genome stability, since long-tract HDR has been associated with increased risk of genomic instability.

1.3.3 Dominant-negative RAD51 mutants in experimental models

A high frequency of tumors was induced when a dominant-negative RAD51 was expressed in p53-defective Chinese hamster ovary (CHO) cells and subsequently injected into nude mice (Bertrand *et al.*, 2003). The tumors exhibited faster growth,

spontaneous centrosome duplication defects and aneuploidy compared to injection of control CHO cells or CHO cells overexpressing wild type RAD51.

Another dominant-negative RAD51 used in experimental model is the RAD51-K133R (RAD51KR) mutant. This RAD51 mutant has a point mutation at position 133 where a lysine been exchanged for an arganine. The *RAD51KR* mutant is incapable of ATP hydrolysis (Morrison *et al.*, 1999). The mutant can still bind DNA and catalyze strand exchange between homologous DNAs (Chi *et al.*, 2006). However, ATP hydrolysis seems to be required for dissociation of RAD51 from the nucleoprotein filament. Thereby the *RAD51KR* mutant can stabilize the presynaptic filament and enhance homologous pairing (Chi *et al.*, 2006; Forget *et al.*, 2007). In mouse ES cells the hRAD51KR mutant has been shown to exhibit typical characteristics of an HDR mutant (Stark *et al.*, 2002). This DNA repair phenotype was less pronounced in a study where hRAD51KR was expressed in chicken DT-40 cells (Morrison *et al.*, 1999). In human cells the K133R mutant was incapable of DNA repair when the cells lacked endogenous RAD51 (Forget *et al.*, 2007). Taken together this data suggests that RAD51KR behaves as a dominant-negative mutant for DNA repair function in mammalian cells possibly by stalling the dissociation of the RAD51 nuclear filament and thereby reducing the free pool of endogenous RAD51 available for DNA repair.

1.4 BRCA1/BARD1 COMPLEX

An inherited germline mutation in either BRCA1 or BRCA2 dramatically increases the risk to develop ovarian and breast cancer. In these tumors the other BRCA allele is typically lost (Collins *et al.*, 1995; Cornelis *et al.*, 1995). The BRCA1 gene encodes a large protein of 1863 amino acids (Miki *et al.*, 1994). The protein has a RING finger domain in its amino-terminal and tandem BRCT repeats in its carboxy-terminal end. More than half of the protein is encoded by exon 11 that includes a nuclear localization signal (NLS). Many different biological functions have been assigned to BRCA1 such as DNA repair, cell cycle control, apoptosis, centrosome duplication and transcriptional regulation (Jasin, 2002; Moynahan *et al.*, 1999; Moynahan *et al.*, 2001a; Scully *et al.*, 2000; Welcsh and King, 2001).

At the RING finger domain BRCA1 interacts with another RING finger protein, BRCA1-Associated Ring Domain (BARD1). Like BRCA1 BARD1 also contains two

BRCT domains in its C-terminal end. In addition BARD1 also have three tandem ankyrin repeats upstream of the BRCT repeats (Wu *et al.*, 1996). BARD1 interacts with BRCA1 and thereby stabilizes the BRCA1 protein (Hashizume *et al.*, 2001; Joukov *et al.*, 2001; Xia *et al.*, 2003) and the interaction has also been reported to enhance the affinity of BRCA1 for DNA (Simons *et al.*, 2006). Overexpression of BARD1 increases BRCA1 stability and thereby inhibits cell cycle dependent degradation of BRCA1 (Choudhury *et al.*, 2004; Hashizume *et al.*, 2001; Joukov *et al.*, 2001; Xia *et al.*, 2003). Mice null for either BRCA1 or BARD1 show similar phenotype including early embryonic lethality and mouse embryonic fibroblasts (MEFs) generated from these mice display severe chromosome aberrations (Gowen *et al.*, 1996; Hakem *et al.*, 1997; Liu *et al.*, 1996; Ludwig *et al.*, 1997; McCarthy *et al.*, 2003; Snouwaert *et al.*, 1998). The close link between BRCA1 and BARD1 function and the fact that the two proteins are typically found in a complex with each other suggests that the BRCA1-BARD1 heterodimer is the physiological relevant form of BRCA1 (Yu and Baer, 2000).

1.4.1 BRCA1/BARD1, ubiquitin ligase activity

Apart from stabilizing BRCA1 the BRCA1-BARD1 heterodimeric RING finger also acquires E3 ubiquitin ligase activity in an E2-dependent manner (Hashizume *et al.*, 2001). This ligase activity is abolished by common tumor associated mutations in the RING finger of BRCA1 (Brzovic *et al.*, 2003; Hashizume *et al.*, 2001; Lorick *et al.*, 1999; Ruffner *et al.*, 2001), strongly suggesting a tumor suppressive function for the BRCA1-BARD1 complex. Cells carrying these BRCA1 mutations are hypersensitive to irradiation (Ruffner *et al.*, 2001), implying a role for the ubiquitin ligase activity in the DNA damage repair function by the complex.

The most common function of polyubiquitylation is targeting proteins for degradation by isopeptide linkage using lysine residues of ubiquitin to form polypeptid chains on the targeted protein. The most common lysine used to build these chains is K48. However, the E3-ligase activity by the BRCA1-BARD1 heterodimer primarily directs polymerization of ubiquitin by the less common K6 lysine (Wu-Baer *et al.*, 2003), which is an indication that the E3-ligase activity by the heterodimer is not primarily degradation. At sites of replication stress or DNA damage the BRCA1-BARD1 heterodimer is auto-ubiquitylated by forming K6-linked ubiquitin structures (Morris

and Solomon, 2004), which in turn increases the ligase activity of the complex 20-fold (Chen *et al.*, 2002; Mallery *et al.*, 2002; Wu-Baer *et al.*, 2003).

It has also been shown, *in vitro*, that the BRCA1-BARD1 heterodimer can ubiquitylate components of the RNA Pol II complex after DNA damage and thereby controlling cell-cycle progression by degrading these components (Kleiman *et al.*, 2005; Starita *et al.*, 2005). It has also been reported that the E3 ligase activity of the BRCA1-BARD1 complex can monoubiquitylate histone H2AX, which implies a role in chromatin remodeling by the complex (Chen *et al.*, 2002; Mallery *et al.*, 2002).

Another study proposes that the E3 ligase activity of the BRCA1-BARD1 complex can ubiquitylate centrosome components, including γ-tubulin (Starita *et al.*, 2004). Inability to do so led to centrosome amplification. This taken together strongly supports the importance of the ubiquitin ligase activity of the heterodimer in DNA repair.

1.4.2 BARD1 in DNA repair

Paper I in this thesis is the first article showing experimental evidence that BARD1 is important in BRCA1-mediated DSB repair by the HDR pathway (Westermark *et al.*, 2003). As described above, the acquired E3-ubiquitin ligase activity of the BRCA1-BARD1 heterodimeric RING finger appears to play an important role in DNA repair function. Furthermore, it has recently been shown that all motifs in BARD1 are essential for repair of an induced DSB by HDR (Laufer *et al.*, 2007). Deletion of either the RING domain, the ankyrin repeats or the BRCT repeats of BARD1 leads to a decrease in DNA repair efficiency (Laufer *et al.*, 2007). In addition, deletion of the ankyrin or the BRCT repeats led to chromosomal instability when expressed in a Bard-null mammary carcinoma cell line (Laufer *et al.*, 2007). Mutations of putative phosphorylation sites within the BRCT repeats in BARD1 did not ablate the DNA repair ability of BARD1 neither did cancer-associated missense mutations in this area (Laufer *et al.*, 2007; Westermark *et al.*, 2003).

It has been shown that BARD1 can be phosphorylated after DNA damage on serine and threonine residues located within the BRCT repeats (Kim *et al.*, 2006). The phophorylation of BARD1 was suggested to be dependent on the phophoinositide 3-kinase-related protein kinase (PIKK) family and also on BRCA1 interaction (Kim *et*

al., 2006). Mutations of these phosphorylation sites abrogated the inhibition of polyadenylation and degradation of RNA polymerase II after DNA damage (Kim *et al.*, 2006). This indicates that phosphorylation of BARD1 is of importance in response to DNA damage. Since different phosphorylation sites were mutated in the work of Laufer *et al.* (Laufer *et al.*, 2007) compared to the study by Kim *et al.* (Kim *et al.*, 2006) these results does not necessarily contradict each other.

It has recently been shown that the BRCT domains of BRCA1 can interact with the ubiquitin-binding protein RAP80 (Kim *et al.*, 2007; Sobhian *et al.*, 2007; Wang *et al.*, 2007). RAP80 recruits the BRCA1-BARD1 complex to ubiquitin structures at sites of DSBs. In addition, interaction with BARD1 enhances the DNA binding ability of BRCA1 (Simons *et al.*, 2006).

1.4.3 BARD1 in tumorigenesis

The close association between BRCA1 and BARD1 has rendered the interest to study possible BARD1 mutations in breast and ovarian cancer patients. So far no deleterious mutations of BARD1 have been found. However, several point mutations have been identified, two of them was also found in the germ line of cancer patients (Ishitobi *et al.*, 2003; Thai *et al.*, 1998). One of the germ line mutation, Gln564His, was observed in a patient diagnosed with sporadic endometrial, breast and ovarian cancer. In at least one of these tumors the wild type allele was lost. This mutation was not found in any of the normal tissues tested (Thai *et al.*, 1998). Cells with the Gln564His mutation have been shown to have defect in polyadenylation in response to DNA damage (Kleiman and Manley, 2001) and it is also defective in apoptotic activity (Irminger-Finger *et al.*, 2001).

In several studies an elevated frequency of a Cys557Ser mutation in patients with breast and ovarian cancer was reported (Ghimenti *et al.*, 2002; Karppinen *et al.*, 2006; Karppinen *et al.*, 2004; Sauer and Andrulis, 2005; Stacey *et al.*, 2006). The highest prevalence of this mutation was found in breast cancer patients without a family history of ovarian cancer (Karppinen *et al.*, 2004). The Cys557Ser mutation were also observed in two other studies, but in these studies were conceived as a normal polymorphism (Ghimenti *et al.*, 2002; Thai *et al.*, 1998). Interestingly, Gln564His and Cys557Ser mutations are found in close proximity to each other in a region of the

BARD1 protein proposed to be crucial for apoptosis (Feki *et al.*, 2005; Feki *et al.*, 2004; Jefford *et al.*, 2004). Thus, the apoptotic function of BARD1 might be of importance in preventing tumor formation.

BARD1 has also been shown to be overexpressed in the cytoplasm of cells in breast and ovarian cancers (Wu *et al.*, 2006). A majority of these tumors expressed a truncated BARD1 protein lacking the 5' RING domain. This overexpression was associated with high malignancy and poor prognosis (Wu *et al.*, 2006). Different BARD1 isoforms, with possible oncogenic potential, has also been reported (Li *et al.*, 2007). Suggesting a role for aberrant BARD1 expression in tumor progression.

1.4.4 BRCA1 independent role of BARD1

There are several reports suggesting a BRCA1 independent role for BARD1 in apoptosis (Feki *et al.*, 2005; Feki *et al.*, 2004; Irminger-Finger and Leung, 2002; Irminger-Finger *et al.*, 2001; Jefford *et al.*, 2004; Rodriguez *et al.*, 2004). Overexpression of BARD1, both *in vivo* and *in vitro*, shows an increase in cell death, with typical characteristics of apoptosis. Moreover, BARD1-repressed cells have been shown to be deficient in apoptosis after genotoxic stress (Irminger-Finger *et al.*, 2001). BARD1 expression levels are increased in apoptotic cells and a significant shift from the nucleus to the cytoplasm has been noted (Jefford *et al.*, 2004; Rodriguez *et al.*, 2004). It has been proposed that BARD1 induces apoptosis by binding, stabilizing and phosphorylating p53 on serine-15 in the cytoplasm (Feki *et al.*, 2005; Irminger-Finger *et al.*, 2001). The apoptotic function of BARD1 appears to be dependent on functional p53, but independent of BRCA1 (Feki *et al.*, 2005; Irminger-Finger *et al.*, 2001). BRCA1 expression seems even to inhibit the apoptotic function of BARD1 (Irminger-Finger *et al.*, 2001; Rodriguez *et al.*, 2004). It has recently been shown that cytoplasmic BARD1 can localize to the mitrochondria and contribute to oligomerization of the pro-apoptotic factor Bax, thereby inducing apoptosis. BRCA1 was not required for the mitochondrial localization of BARD1 (Tembe and Henderson, 2007). On the other hand, it has also been suggested that BARD1 can have an inhibitory effect on apoptosis by inhibiting BRCA1 induced apoptosis by nuclear retention of BRCA1 (Fabbro *et al.*, 2004).

A plausible model based on these data could be that BARD1 can direct the cells fate after genotoxic stress either to DNA-repair by binding and stabilizing BRCA1 in the nucleus, or induce apoptosis by binding and stabilizing p53 in the cytoplasm.

1.5 DNA DAMAGE RESPONSE IN TUMORIGENESIS

Activation of DNA damage response (DDR) proteins is rarely found in normal tissues. The only tissues, which scored positive for these proteins, are tissues that undergo genome rearrangements and meiotic recombination, *i.e.* lymphocytic cells in the bone marrow and spermatocytes in adult testes (Bartkova *et al.*, 2005a). However, upregulation of DDR genes have been found in pre-malignant lesions of colon, urinary bladder, skin, prostate and lung (Bartkova *et al.*, 2005b; Bartkova *et al.*, 2007; Bartkova *et al.*, 2006; Di Micco *et al.*, 2006; DiTullio *et al.*, 2002; Fan *et al.*, 2006; Gorgoulis *et al.*, 2005; Nuciforo *et al.*, 2007; Tort *et al.*, 2006), while corresponding malignant lesions showed significantly lower frequency in expression of these proteins. This suggests that DDR could serve as an inducible barrier to prevent tumor formation by inducing cell death or cellular senescence. Defects in genes involved in DDR, for example p53, ATM, 53BP1 and BRCA1 and 2, could lead to escape from check point control and subsequently lead to chromosomal rearrangements and genetic instability (Bartek *et al.*, 2007a; Bartek *et al.*, 2007b), typical hallmarks of cancer (Hanahan and Weinberg, 2000).

Uncontrolled growth caused by upregulation of onogenes and downregulation of tumor suppressor genes is another hallmark of cancer (Hanahan and Weinberg, 2000). Studies in cell culture and mouse models have shown that overexpression of a plethora of oncogenes can cause DNA damage checkpoint activation, e.g. H-ras, mos, cyclin E, Cdc25A, E2F1, cdc6, STAT5 and cMyc (Bartkova *et al.*, 2005b; Bartkova *et al.*, 2006; Di Micco *et al.*, 2006; Frame *et al.*, 2006; Mallette *et al.*, 2007; Powers *et al.*, 2004; Pusapati *et al.*, 2006; Reimann *et al.*, 2007; Tort *et al.*, 2006). Inactivation of the tumor suppressor pRb has also been shown to activate DDR (Pickering and Kowalik, 2006; Tort *et al.*, 2006), while inactivation/activation of other proteins in the pRB pathway, p16ink4a and cyclinD1, did not (Tort *et al.*, 2006).

Even a combination of growth factors (basic fibroblast growth factor, stem cell factor and endothelin-3) injected into human skin xenografts in a mouse model for skin

hyperplasia could induce DDR (Gorgoulis *et al.*, 2005). In this model it was shown that expression of growth factors could induce genomic instability at fragile sites a few weeks after induction of hyperplasia, suggesting that the observed instability was an early event in this tumor model (Gorgoulis *et al.*, 2005). This data corresponds well with results in paper II in this thesis. In paper II we show that by overexpressing the growth factor PDGFB in the brain of newborn mice we can induce hyperplastic lesions within three weeks and that these lesions are aneuploid (Westermark *et al.*, 2008). This implies that the genomic instability seen in these models might primarily be caused by replication stress caused by overexpression of oncogenes, independent of inactivation of care-taker genes. This is further supported by our data showing that inactivation of $p19^{Arf}$ had no apparent effect on genomic instability (Westermark *et al.*, 2008). It also implies that genomic instability can precede the malignant transformation rather than being a consequence of tumor progression.

The proposed model for this phenomenon is that overexpression of an oncogene will cause replicative stress, including stalled replication forks, collapse of the forks and subsequent DNA damage that will activate DDR and check point control. In cells with functional cell cycle regulation this can either lead to cell death or cell cycle arrest. If the cell cycle block becomes permanent it can be manifested as oncogene-induced senescence (OIS) (Bartkova *et al.*, 2006; Di Micco *et al.*, 2006). Thus, activation of DDR will prevent tumorigenesis. However, this oncogenic stress can also induce genomic instability (Gorgoulis *et al.*, 2005; Westermark *et al.*, 2008), which subsequently could lead to tumor progression.

1.6 DNA DAMAGE PATHWAYS AS A POSSIBLE TARGETS FOR CANCER THERAPY

Conventional cancer therapy typically involves different types of DNA damaging agents, such as irradiation and chemotherapy. Even though these types of treatments are rather efficient in many types of cancers it also have serious side effects, since they target all proliferating cells and not only the tumor cells. Moreover, it has become evident that tumor cells can evade this treatment by deregulation of DNA repair pathways. Increased activation of these pathways can potentially overcome DNA damage activated cell cycle checkpoints and allow cells that should have been eliminated to continue proliferation. This could in turn lead to increased genomic instability and tumor progression. Apart from overexpression of DNA repair proteins in

tumors, persons with hereditary mutations in DNA repair genes are predisposed to tumor development, e.g. *BRCA1/2*, *FANC* genes, *ATM, BLM* (Bloom's syndrom) and *WRN* (Werner syndrome). Pre-clinical models has shown that inhibitors of DNA repair can enhance the efficacy of DNA damaging anti-cancer drugs. Furthermore, recent findings indicate that inhibitors of DNA repair also could be used as single agents to treat patients with known defects in DNA repair pathways. Inhibitors of different DNA damage pathways are currently being tested in clinical trials (Helleday *et al.*, 2008).

To study cells sensitivity to DNA damaging treatment, RAD51 has been downregulated in a multitude of ways and in a variety of cell types. Downregulation of RAD51 by expressing a RAD51 ribozyme mini gene led to increased sensitivity to γ-irradiation in a prostate tumor cell line (Collis *et al.*, 2001). Sensitivity to γ-irradiation was also increased in mouse cells when Rad51 was knocked down by antisense nucleotides (ODNs) (Taki *et al.*, 1996). A combination of anti-sense RAD51 and low dose irradiation increased survival in a mouse glioma model (Ohnishi *et al.*, 1998). RAD51 siRNA has been shown to enhance the effect of radiation and cisplatin in a variety of cancer cells both *in vitro* and *in vivo* (Ito *et al.*, 2005; Saydam *et al.*, 2007). It has also been shown that the tyrosine kinase inhibitor, Gleevec/Imatinib, can downregulate RAD51 in glioma cell lines and thereby sensitize the cells to radiation (Russell *et al.*, 2003), implying a link between growth factor signaling and RAD51 regulation.

Other members of the HDR network has also been investigated as potential targets for cancer therapy. Peptide-based inhibition of the RAD51 paralog RAD51C in CHO cells reduced DNA-damage induced foci and increased the cells sensitivity to cisplatin (Connell *et al.*, 2004). In another study, the ATM inhibitor caffeine sensitized glioma cells to irradiation (Short *et al.*, 2007).

Inhibitors of DNA repair have also been shown to work as single agents. Inhibition of poly (ADP-ribose) polymerase (PARP) can specifically kill cells with deficiency in proteins involved in the HDR pathway (e.g. BRCA1/2, RAD51, ATM, ATR, CHK1, CHK2, RPA1, NBS1, FANCA/C/D2) (Bryant *et al.*, 2005; Farmer *et al.*, 2005; McCabe *et al.*, 2006). However, cells deficient for RAD52 were not sensitive to PARP inhibition, implying that the PARP inhibition primarily effected repair by gene conversion and not single strand annealing (SSA) (McCabe *et al.*, 2006). These results

imply the possible benefit of PARP inhibitors for treating tumors with defects in proteins involved in HDR.

2 GLIOMAGENESIS

2.1 PDGF AND THE BRAIN

2.1.1 PDGF ligands and receptors

Platelet-derived growth factor (PDGF) was first identified as a serum component important for proliferation of arterial smooth muscle cells (Ross *et al.*, 1974). Later it was found that PDGF had specific receptors, primarily on cells derived from connective tissue and from normal and malignant glia (Heldin *et al.*, 1981). At present four family members of PDGF is known: PDGF-A, -B, -C and D. They can form homo- and heterodimers (AA, AB, BB, CC and DD) and they bind to the tyrosine kinase receptors PDGFRα and PDGFRβ. The PDGF receptors can also form homo- and heterodimers (αα, αβ and ββ). The different PDGF ligands have different affinity for the PDGF receptors: AA binds αα, AB and CC binds αα and αβ, BB binds all three receptors and DD binds αβ and ββ. Upon binding the PDGF receptors dimerize and are activated by cross phophorylation of their intracellular tyrosine kinase domains. The phophorylated tyrosine residues serves as docking sites for several different proteins involved in mitogenic signaling cascades. These signaling pathways includes the Ras-Mitogen Activated Protein Kinase (MAPK), STAT transcription factor, phospholipase Cγ (PLCγ), Src family kinase and phosphatidylinositol 3-kinase (PI3K) pathways, which in turn activate downstream proteins such as Akt, Ras, Rho and Jak kinases. This suggests diverse functions of the PDGF family and also that the different binding combinations of the PDGF family members serve different cellular functions. (Shih and Holland, 2006; Yu *et al.*, 2003).

2.1.2 PDGF in brain development

The central nervous system (CNS) stem cell gives rise to neuronal and glial progenitors, which subsequently give rise to the mature cell types of the CNS: neurons, oligodendrocytes, astrocytes and ependymal cells. This maturation process is tightly regulated by the expression of different growth factors and the different maturation stages have been defined by cell morphology and expression of specific markers (Shih and Holland, 2006).

In the adult brain the largest germinal center and source of neural stem cells (B-cells) is in the subventricular zone (SVZ) in the lateral ventricle, which give rise to mature

neurons and oligodendrocytes (Menn et al., 2006). B-cells express PDGFRα and it has been shown that PDGFRα is important for oligodendrogenesis from these cells (Jackson *et al.*, 2006). PDGFA is also expressed in the SVZ, while PDGFB is primarily expressed in the cortex. The importance of PDGF in regulation of B-cells in the SVZ was further emphasized by a study showing that forced expression of PDGFA in mouse SVZ increased PDGFRα phosphorylation and proliferation of B-cells, leading to hyperplasia. These hyperplasias were dependent on PDGFA stimulation (Jackson *et al.*, 2006). The same study also showed phosphorylation of PDGFRα in the SVZ in human adult brain, suggesting that endogenous PDGF signaling occur not only during development but also in the adult brain (Jackson *et al.*, 2006).

PDGFB and PDGFRβ are important for vascular development in the mouse. Endothelial cells produce PDGFB and PDGFRβ is expressed by vascular smooth muscle cells, also called pericytes (Holmgren *et al.*, 1991; Lindahl *et al.*, 1997). Deletion of either of these genes causes embryonic lethality in mice due to microvascular hemorrhage and edema, caused by lack of pericytes (Hellstrom *et al.*, 2001; Kaminski *et al.*, 2001; Leveen *et al.*, 1994; Lindahl *et al.*, 1997). The pericytes surrounds and protects the endothelial cells in the blood vessel (Sims, 1986). This strongly suggests a paracrine loop between PDGFB ligand on endothelial cells in blood vessels and PDGFRβ on pericytes during angiogenesis. It has also been shown that PDGF ligands and receptors often are co-expressed on tumor cells suggesting an important role for autocrine/paracrine stimulation by PDGFs in the development of tumors (Hermanson *et al.*, 1992; Uhrbom *et al.*, 2000; Westermark *et al.*, 1995).

2.2 BRAIN TUMORS

Similar to normal brain, primary brain tumors are composed of multiple cell types: neurons, glia, smooth muscle and endothelial cells based on the similarity to their normal counterparts in morphology and expression of cell-type specific markers. Brain tumors are also composed of cells of various differentiation stages of each particular cell type (Collins, 2004; Ohgaki and Kleihues, 2005).

Gliomas are classified into different grades (I-IV) based on malignancy according to the World Health Organization (WHO), where glioblastoma multiforme (GBM) is classified as the most malignant form (grade IV). Gliomas grow diffusely and infiltrate

the surrounding tissue and are therefore very challenging to surgically remove. The prognosis for glioma patients is poor and only a minority of patients reaches long-term survival. Low-grade gliomas are divided in to two groups: astrocytomas and oligodendrogliomas. Diffuse lower grade tumors have a strong tendency for malignant progression and commonly recur as a GBM in the proximity of the primary tumor location within a few years after surgery. The majority of patients diagnosed with GBM die within a year of diagnosis (Holland, 2001; Ohgaki and Kleihues, 2005; Rich and Bigner, 2004).

It has previously been believed that gliomas arise from glial or neural progenitors, since cells in glioma mostly resemble immature astrocytes and/or immature oligodendrocytes. Another possible explanation could be that mature astrocytes and oligodendrocytes dedifferentiate due to the genetic changes they encounter during tumor development. However, in recent years the cancer stem cell hypothesis has evolved suggesting that only a minority of tumor cells have the capacity to self-renew and give rise to new tumors (Fomchenko and Holland, 2005).

Brain tumor stem cells have been isolated from human brain tumors based on their expression of the surface antigen CD133. These cells could form neurospheres in culture, were able to self-renew indefinitely under the right conditions and could initiate glioma-like tumors in serial-transplantations in mice (Galli *et al.*, 2004; Singh *et al.*, 2003). Only CD133 positive cells from human tumors had the capacity to generate tumors in mice that closely resembled the original tumor. CD133 negative cells from the same tumor were not able to establish tumors. This supports the idea that the CD133 positive brain tumor stem cell might be the initiating cell in gliomagenesis (Singh *et al.*, 2004).

It has also been shown that injection of a retroviral *PDGFB-GFP* construct into the brain of adult rats could induce brain tumors in all animals and with short latency (Assanah *et al.*, 2006). Only a fraction of the tumor cells expressed the GFP-tag used in this study, implying that uninfected cells were recruited to the tumor by paracrine stimulation by PDGFB. This was further supported by the recruitment of perivascular smooth muscle cells to the tumor, possibly by activation of the PDGFRβ. However, PDGFRβ expression in the vasculature was not investigated in this study. Both GFP positive and negative cells in the tumors expressed glial progenitor cell markers such as

nestin, olig2, NG2 and PDGFRα, but not the astrocytic marker GFAP suggesting that these tumors had arised from white matter progenitors (Assanah *et al.*, 2006). It has also been shown using the RCAS/tv-a model system that PDGFB can induce tumors from both nestin and GFAP expressing cells (Dai *et al.*, 2001; Dai *et al.*, 2005; Shih *et al.*, 2004; Tchougounova *et al.*, 2007). It is noteworthy that brain tumors in humans can occur throughout the whole brain and not only in the vicinity of the SVZ where the majority of normal neural stem cells reside. Thus, the cell-of-origin in gliomas remain elusive. However, one hypothesis does not necessarily overrule the other.

2.2.1 Genetic changes in glioma

Gliomas are markedly heterogeneous tumors, even within the same tumor. Despite the heterogenous appearance of gliomas, the typical genetic targets for alteration are within specific cellular processes, primarily in two main functional groups: activation of receptor tyrosine kinase (RTK) signaling pathways and loss of cell cycle regulation. It appears that modifications in both pathways are required for gliomas to occur. Common alterations in the RTK signaling pathways are overexpression of growth factors (e.g. *PDGF*, *FGF*) and overexpression/amplification of tyrosine kinase receptors (e.g. *PDGFR*, *EGFR*). An excess of growth factors and their receptor counterpart is frequently seen in the same cell, which can lead to autocrine stimulation and increased activity of down-stream effectors in the same pathway. Activation of the RTK pathway is typically accompanied by inactivation of the p53 pathway (e.g. *p53, p14*^{ARF}) and/or the RB pathway (e.g. *RB, p16*^{INK4a}). Not surprisingly, the number of genetic alterations often correlates with tumor grade, where an increase in gene amplifications and deletions give rise to tumors of higher grade (Collins, 2004; Ohgaki and Kleihues, 2005; Rich and Bigner, 2004; Sanson *et al.*, 2004).

2.2.2 Glioma therapy

Gliomas are often resistant to radiation therapy. It would therefore be of great benefit for treatment of gliomas to sensitize the tumor cells to radiotherapy. Downregulation of proteins involved in DNA repair has proven to be quite efficient in radiosensitizing tumor cells and tumors in animal models (Bao *et al.*, 2006; Golding *et al.*, 2007; Ohnishi *et al.*, 1998; Russell *et al.*, 2003; Saydam *et al.*, 2007; Short *et al.*, 2007). It has been suggested that the CD133 positive brain cancer stem cells promotes radioresistance in glioma by activating DDR. In a study by Bao *et al.* they showed that

CD133 positive cells more efficiently activated DDR in response to radiation compared to the CD133 negative cells and that the pool of CD133 positive cells increased after the radiation treatment (Bao *et al.*, 2006). Targeting DNA repair pathways, especially the HDR pathway, could be of special gain in brain tumors since in the majority of normal post-mitotic neuronal cells DNA repair is diminished and the treatment would thereby specifically target the tumor cells. However, the benefits of such combined therapy in the clinic remain to be shown.

The most efficient therapy to treat gliomas, at present, is treatment with the alkylating agent, Temozolomide. However, many gliomas are resistant to Temozolomide by expressing the DNA repair enzyme, O^6-Methylguanine-DNA-Methyltransferase (MGMT). Several different strategies have been developed to silence MGMT and thereby improve treatment by Temozolomide (Esteller *et al.*, 2000; Gerson, 2004; Hegi *et al.*, 2005; Stupp *et al.*, 2005).

2.3 ANIMAL MODELS OF GLIOMAGENESIS

To further understand the molecular mechanisms behind gliomagenesis several mouse models have been developed. Models where brain tumors are induced by somatic-cell gene-transfer have proven to be very informative. The genes are delivered to the brain cells by intracerebral injection of oncogene-carrying retroviruses. These models allow the tumors to develop from somatic cells in their normal cellular environment and they show striking histopathological similarities with their human counterparts. By injecting different combinations of oncogene-expressing retroviruses in mice with deletions of different tumor suppressor genes it is possible to delineate the impact of various genetic alterations as well as combination of alterations in brain tumor formation (Uhrbom and Holland, 2001).

2.3.1 PDGFB/MMLV

The *PDGFB*/MMLV (Moloney murine leukemia virus) model system was developed to investigate the role of *PDGFB* in brain tumorigenesis. *PDGFB* expressing retrovirus was intracerebrally injected in newborn mice and highly malignant brain tumors of varying histological appearence developed in 40% of the animals. A majority of the tumors showed characteristics of human gliomas and all of them co-expressed *PDGFB* and *PDGFRA* mRNA, suggesting a critical role for PDGF in tumor initiation. It was

also shown that a cell line established from these tumors was dependent on autocrine PDGF stimulation for its proliferation (Uhrbom *et al.*, 1998). However, it is known that PDGF overexpression and autocrine stimulation alone is not sufficient to cause glioma formation in humans. It was therefore hypothesized that additional mutations were caused by retroviral insertional mutagenesis to induce these highly malignant tumors. The PDGFB-induced tumors were investigated for proviral tagging of cellular genes. Several known and novel genes of tumor progression were tagged multiple times in the analyzed tumors. Of special interest for this thesis is that two genes associated with HDR were identified, the *Rad51* paralog *Rad51b* and *Fancc*, which were tagged in two different tumors respectively. *Rad51b* displaying elevated expression similar to the *Pdgfrα* expression in these tumors (Johansson *et al.*, 2004; Johansson *et al.*, 2005). This offers the possibility that abnormalities in the HDR pathway could contribute to progression of *PDGFB* induced gliomagenesis.

2.3.2 RCAS/t-va

The receptor for MMLV is expressed in many different cell types in the mouse and produced virus can thereby infect a variety of cell types when injected into the brain of newborn mice. To address the question of cell-of-origin in gliomagenesis as well as the impact of combinations of different genetic alterations, the RCAS/tv-a model system was developed (Holland *et al.*, 1998a; Holland *et al.*, 1998b). The model is based on replication competent ALV splice acceptor (RCAS) and transgenic mice expressing the receptor for RCAS, *tv-a*. The *tv-a* receptor is normally only expressed in avian cells. This makes it possible to engineer transgenic mice to express the *tv-a* receptor from cell specific promoters and the expression of the RCAS virus will thereby only be directed to cells that express this promoter. The RCAS virus is replication incompetent in mammalian cells (Holland *et al.*, 1998a; Holland *et al.*, 1998b; Uhrbom and Holland, 2001).

Two transgenic *tv-a* mouse lines have been established to study gliomagenesis, one expressing *tv-a* from the nestin promoter (*Ntv-a*) and the other expressing *tv-a* from the GFAP promoter (*Gtv-a*). In the *Ntv-a* transgenic mouse the RCAS vector will deliver genes by infecting neural progenitors and in *Gtv-a* mice the genes carried by the RCAS vector will be transferred to astrocytes (Uhrbom and Holland, 2001). It is worth noting that neural stem cells in the SVZ (B-cells) also express GFAP (Jackson *et al.*, 2006). Hence, the RCAS virus can also target these cells in the *Gtv-a* mice. The RCAS/tv-a

mice can be crossed with mice with targeted deletions of different tumor suppressor genes (Holland *et al.*, 1998a; Holland *et al.*, 1998b; Tchougounova *et al.*, 2007; Uhrbom *et al.*, 2002; Uhrbom *et al.*, 2005; Uhrbom *et al.*, 2004). The impact of different gene alterations on brain tumorigenesis can be studied by intercerebral injection in newborn mice of RCAS producing cells that express a gene of interest, by itself or in combination with other RCAS producing cells. This model offers a possibility to study a variety of combination of genetic alterations in specified cell types (Uhrbom and Holland, 2001).

The RCAS/tv-a mouse model has been extensively used to elucidate the role of different oncogenes and tumor suppressor genes in brain tumor formation, by themselves or in various combinations. In the first study using the RCAS/tv-a model system the *EGFR* was overexpressed. Overexpression of *EGFR* alone in wild type or *p53*-deficient *Ntv-a* mice did not induce tumors, but when expressed in an *Ink4a-Arf* null background a high frequency of tumors developed (Holland *et al.*, 1998a).

Overexpression of *PDGFB* in *Ntv-a* as well as in *Gtv-a* mice induces a large number of tumors, similar to what has previously been seen in the *PDGFB*/MMLV gene transfer model, however, the tumors where primarily oligodendrogliomas (*Ntv-a*, *Gtv-a*) and mixed oligoastrocytomas (*Gtv-a*) (Dai *et al.*, 2001). Thus, when *PDGFB* is directed to specific cell types it gives a more defined tumor population. *PDGFB* was also overexpressed in an *Ink4a-Arf* null background, the incidence in *Ntv-a* mice was similar to wild-type mice but the in the *Gtv-a* mice tumor incidence was elevated almost 2-fold. The tumors were more malignant and had shorter latency in both *Ntv-a* and *Gtv-a* mice. Of note, this study demonstrated that overexpression of *PDGFB* in *p53*-null animals produced similar result to wild type animals (Dai *et al.*, 2005). This is consistent with the fact that *TP53* mutations rarely occur in oligodendrogliomas, while silencing of the *Ink4a-Arf*-locus by hypermethylation is quite frequent (Collins, 2004). By elevating the PDGFB levels, tumors of higher grade and with increased vascularization was induced. Tumor maintenance was dependent on PDGFB (Shih et al., 2004).

The respective roles of the two members of the *Ink4a-Arf* locus, $p16^{INK4a}$ and $p14/p19^{ARF}$, in PDGFB-induced gliomagenesis have also been investigated (Tchougounova *et al.*, 2007). From this study it was found that the two tumor

suppressors had differential roles in a cell type specific manner in this tumor model system. Both tumor suppressors induced a significant increase in tumor incidence compared to wild type in the *Gtv-a* mice and the *Ink4a*-null background increased tumor initiation compared to the other genotypes. Tumor incidence in *Ntv-a* mice was only slightly increased by the tumor suppressor loss. However, *Arf*-loss had a pronounced effect on malignancy compared to *Ink4a*-loss, in both *Gtv-a* and *Ntv-a* mice. Based on *in vitro* data in primary brain cultures it was suggested that the increase in tumor malignancy in the *Arf*-null background was through deregulation of MAPK pathway (Tchougounova *et al.*, 2007).

In paper II we could confirm that *Arf*-loss led to increased malignancy in PDGFB-induced gliomagenesis, using *Ntv-a* mice. The most malignant tumors in this study (grade III) showed significant vascularization and this vasculature clearly expressed PDGFRβ, while the tumor cells expressed high levels of PDGFRα, suggesting autocrine/paracrine PDGFB stimulation in the formation of these tumors. No grade III tumors or extensive vascularization was seen in tumors in the wild type background (Westermark *et al.*, 2008). It has been shown that *Arf* play an important role in the regulation of perivascular cells, such as pericytes, in the developing eye. Loss of *Arf* led to excessive proliferation of these cells. It was further shown that this hyperproliferation was dependent on PDGFRβ and that Arf negatively regulated PDGFRβ expression independently of p53 and Mdm2 (Silva *et al.*, 2005; Thornton *et al.*, 2005). These results offer a rather persuasive explanation why *Arf*-loss contributes to malignant progression in PDGFB-induced tumorigenesis, but not in *Ink4a*- and *p53*-null animals. However, it has also been shown that *Arf* is the major tumor suppressor in *Gtv-a* and *Ntv-a* mice in *K-ras* induced gliomagenesis (Uhrbom *et al.*, 2005), suggesting an additional and more general tumor suppressor function for *Arf* in the brain.

3 AIM OF THESIS

The overall aim of this thesis was to investigate the role of HDR in tumor initiation, tumor progression and genetic instability. The specific aims were:

- To investigate whether BARD1 was involved in DNA repair by HDR of DSBs and whether the interaction between BARD1 and BRCA1 was essential for the two proteins role in HDR. (Paper I)

- To elucidate whether a potential cancer causing germline mutation in BARD1 gave rise to repair defects in the HDR pathway. (Paper I)

- To study the effect of overexpression of either wild type RAD51 or a dominant negative RAD51 on PDGFB-induced brain tumor formation in wild type and *Arf-/-* mice. (Paper II)

- To identify the responsive element/elements for IL-1β downregulation of *PDGFRA* in the promoter region of *PDGFRA*. (Paper III)

4 RESULTS

4.1 PAPER I

To investigate whether the BRCA1 interacting protein BARD1 is important in HDR of a DSB we took a dominant negative approach. For this study we used wild type mouse embryonic stem (ES) cells and Brca1-deficient ES cells with an integrated repair substrate, *DR-GFP*. The Brca1-deficient cells express an alternative spliced Brca1 that skips exon 11, Brca1$^{\Delta 11}$. This cell line has previous been shown to have defects in HDR of an induced DSB and is hypersensitive to DNA-damaging agents such as mitomycin C (MMC) (Moynahan *et al.*, 2001a). To elucidate the role of BARD1 in HDR we expressed either full length BARD1 or a truncated BARD1 with an intact N-terminal RING domain but with deleted C-terminal structural motifs in wild type and Brca1-deficient ES cells. We confirmed by two-hybrid analysis and immunoprecipitation that this truncated BARD1 could interact with the endogenous Brca1 and Brca1$^{\Delta 11}$.

We could show that transient expression of the truncated BARD1 construct decreased HDR of an induced DSB in wild type ES cells. The effect of the dominant negative BARD1 was even more pronounced in Brca1-deficient ES cells. Expression of full length BARD1 had no effect on repair in either wild type ES cells or Brca1-deficient ES cells.

To examine if the observed repair defect was dependent on the BARD1 constructs interaction with the endogenous Brca1, two different point mutations were introduced in the RING domain of the truncated BARD1, C83G and L107P. Missense mutations in key cyctein residues in BRCA1 are associated with human tumors and mutations in these residues have also been shown to interfere with RING interacting proteins (Castilla *et al.*, 1994; Friedman *et al.*, 1994). The C83G point mutation in BARD1, however, did not interfere with the heterodimerization between BRCA1 and BARD1. The expression levels of this mutant were significant lower compared to the truncated BARD1 without the point mutation and the effect on HDR were intermediate, probably due to the low expression level of the C83G mutant. The L107P mutant was constructed to completely abolish the interaction between the BARD1 construct and endogenous Brca1. This was confirmed by two-hybrid analysis. No significant decrease

in HDR was observed when this construct was expressed in wild type and Brca1-deficient cells.

To confirm that the repair defects observed were not due to improper cellular localization of either the transfected BARD1 constructs or the endogenous Brca1, immunoprecipitation and cellular fractionation was performed on transfected ES cells. We could show that the transfected BARD1 construct was predominantly nuclear and that Brca1 and Brca1$^{\Delta 11}$ were exclusively nuclear in the BARD1 transfected ES cells. These data support the interpretation that the HDR defect is a direct dominant negative effect due to heterodimer formation between endogenous Brca1 and truncated BARD1 and not due to mislocalization of either of the proteins.

At the time of this study only one potential cancer causing germline mutation in BARD1 had been reported, Gln564His (Q564H) (Thai *et al.*, 1998). We wanted to test whether expression of a full length BARD1 with the Q564H mutation would cause a HDR defect. Our data show that expression of BARD1 with the Q564H mutation had no effect on HDR in our model system.

From this study we conclude that BARD1 is involved in DNA repair by HDR of a DSB. Expression of a dominant negative BARD1 decreases HDR and this decrease is dependent on the heterodimer formation between BARD1 and Brca1. Our result suggests the BRCA1-BARD1 hetrodimer as the functional unit for HDR. We could also show that a reported germline mutation in BARD1 had no effect on HDR in this model system.

4.2 PAPER II

The aim of this study was to investigate the effect of deficient DNA repair in PDGFB-induced gliomagenesis in wild type and Arf-null *Ntv-a* mice. For this study we used the RCAS/tv-a mouse model system. The model is based on the RCAS retroviral vector and transgenic mice expressing the receptor for RCAS, tv-a, from a cell type specific promoter. For our study we used the nestin promoter tv-a (*Ntv-a*) mouse. These mice only express the tv-a receptor in nestin positive cells, i.e. neural progenitor cells. Tumors can be induced by injection of cells expressing various RCAS virus constructs intracerebrally in newborn tv-a expressing mice. It has previously been shown that

injection of PDGFB virus causes glioma like brain tumors in *Ntv-a* mice and that *Arf*-loss increase tumor malignancy in these mice.

To investigate the effect of alterations in DNA repair in PDGFB-induced gliomagenesis, we injected cells expressing the central HDR protein RAD51 or a RAD51 mutant, RAD51-K133R, which has a dominant-negative effect on DNA repair function. These RAD51 constructs were expressed by themselves or in combination with PDGFB virus in wild type and *Arf-/- Ntv-a* mice.

Expression of the RAD51 constructs by themselves did not induce brain tumors in the *Ntv-a* mice. However, when co-expressed with PDGFB they where able to suppress PDGFB-induced gliomagenesis. We could show that overexpression of wild type RAD51 suppressed tumor formation in wild type animals and the suppressive effect was even more pronounced in the Arf-null background. The RAD51KR mutant strongly suppressed PDGFB-induced gliomagenesis in wild type animals but not in the *Arf-/-* mice.

Another intriguing finding in this study was that all the PDGFB-induced tumors were aneuploid, independent of genotype, grade or size. Interestingly, co-expression of PDGFB and the two RAD51 constructs could decrease the rate of aneuploidy and could even render diploid tumors.

We conclude that aneuploidy was an early event in PDGF-induced gliomagenesis independent of genotype, tumor grade or tumor size. Co-expression of RAD51 constructs decreased the aneuploidy caused by PDGFB and suppressed tumor development.

4.3 PAPER III

The aim of this paper was to investigate transcriptional regulation of *PDGFRA* by interleukin-1β (IL-1β). In this paper we used the osteosarcoma cell line MG-63. This cell line was previously shown to downregulate *PDGFRA* after stimulation by IL-1β and this regulation was primarily at the transcriptional level.

To find the responsive element/elements for this regulation we used different length of the *PDGFRA* 5´promoter region coupled to the luciferase gene. This enabled us to

measure *PDGFRA* activity by luciferase assay. We also performed bandshift assays to study protein binding to putative responsive DNA sequences in the *PDGFRA* promoter region. We also introduced mutations in the regions of interest in the *PDGFRA* promoter to try to abolish the inhibitory effect by IL-1β on *PDGFRA* promoter activity.

We were able to map an IL-1β responsive area within the *PDGFRA* promoter region. Although we found a strong correlation between the IL-1β effect and binding of CCAT/enhancer binding proteins (C/EBPs) to the promoter region, we could not abolish the inhibitory effect of IL-1β on *PDGFRA* expression by mutation of these regions. However, our data clearly showed that C/EBP is essential for basal *PDGFRA* transcription in MG-63 cells.

5 CONCLUDING REMARKS

Regulation of DNA repair appears to be a double-edged sword in the evolution and treatment of cancer. On one hand upregulation of DNA repair proteins protects cells from malignant transformation, but on the other hand increased DNA repair activity decreases cells sensitivity to DNA damaging drugs and thereby complicate the treatment of cancer. Moreover, deficiency in DNA repair is associated with chromosomal instability and increased risk of tumorigenesis. Taken together, this indicates that proper function of DNA repair pathways is of utmost importance for prevention and in treatment of cancer. Thus, further understanding of how the DNA repair pathways are regulated in normal and malignant cells have the potential to be of great use in the development of new and more efficient treatments of cancer in the future.

6 ACKNOWLEDGEMENTS

This work was carried out at the Department of Onkologi-Pathology, Karolinska Institutet; Memorial Sloan Kettering Cancer Center, New York and Department of Genetics and Pathology, Uppsala Universitet, and was supported by grants from Swedish Cancer Foundation, STINT, UICC, Robert A Lundbergs foundation. I want to express my sincere gratitude to everyone who has supported me during this time. I particular want to thank:

My supervisor **Monica Nistér**, for your encouragement and support over the years and for putting up with my constant absence from the lab.

My co-supervisor **Maria Jasin**, for welcoming me to your lab in New York and for introducing me to the fascinating DNA repair research field.

Gijs Afink, for being a good friend and an excellent teacher, teaching me all I know about cloning.

Mary-Ellen Moynahan, for introducing me to the BRCA1/BARD1 research field and for being the straight-forward kind of person you are.

Lene Uhrbom, my dear friend and collaborator, for your encouragement, efficiency and focus at work and for all the great times outside the lab in Uppsala and New York.

Marianne Kastemar, for your friendship and for teaching me all I need to know about tissue culture, for all your help and smart technical solutions over the years.

Nanna Forsberg, for your good spirits and for all your help with the mice.

Rona Strawbridge, for reading my thesis and correcting my English, for always being up for coffee and for keeping me company in the lab over Easter.

Anders Zetterberg and his research group, with special thanks to **Kerstin Nyström-Nord** for your excellent help with the Feulgen's staining.

Anna Eriksson, for helping me with stainings and teaching me the Ventana.

Juan Casto, for helping me with FACS analysis.

AnnBritt Spåre, for always being so friendly and helpful with administrative stuff.

Janna Paulsson, for charing your excellent protocol for PDGF receptor staining and for being a nightowl and party girl just like me.

All co-authors, for their contribution to this thesis.

My older “brother” **Bengt Westermark,** for your great sense of humor and your ability to make tumorbiology both interesting and understandable without simplifying it.

Marianne Kastemar and **Annika Hermansson** for taking me under your wings when I first came to Tumörbiologen, only 19 years old.

All past and present people at Tumörbiologen/Rudbeck laboratoriet for great times in the lab and for the best department parties ever!

All members of the Jasin lab, especially **Hildur Helgadottir, Nicole Christ, Jeremy Stark, Jin Oh, Marco Bacchi, Debra Alstond** and **Beth Elliot** for making my time in the Jasin lab the best possible.

Past members of the Nistér group, especially **Susanne Heller, Johanna Andrae, Gijs Afink, Inga Hallin, Xiao-Qun Zhang, Adila ElObeid, Ren Zhi-Ping, Eric Bongcam-Rudloff, Wessen Maruwge, Marcus Ismail and Hanna Berglind.**

Present members of the Nistér group, **Tomador Elsir, Rona Strawbrige, Inga Nazarenko, Sanna Hede, Ersen Kavak, Anna Eriksson, Salah Gadalla, Xiaobing He, Mikael Lindstöm, Liu Tong, Inti Peredo-Harvey, Chunde Li, Jian Zhao, Mingqi Qu and Zhuo-Chun Peng**, for creating a fun, positive and friendly atmosphere in the lab and a special thanks to **Christer Eriksson** for inviting us all to your home for BBQs in the summer and apple picking in the fall.

My “Family”, **Lene, Kicki** och **Carolina** for your neverending friendship and support, for all the good times, excellent dinners (except the soya-one) and interesting discussions over the 10+ years we have known each other.

“Matlaget”, **Maja, Lotta, Åsa, Sibel, Anna, Lissan and Nina** for your friendship, good food and great discussions about everything from world peace to how you pronounce the world “risotto”.

Bill, Melissa, **Kiki**, **Maria, Karin** och **Cyril** for your friendship and all the good times we had together in New York.

“The girls”, **Lene, Hildur, Jale and Jen,** for your frienship and for making my New York experience a little bit more “Sex and the cityish”.

Annika, for being such a close and dear friend even though we see each other so seldom.

Katarina, for your friendship and all the great and wild times in Uppsala.

My parents **Eva** and **Leif**, my step-parents **Gunnar** and **Sylvia** and my sisters **Sara** and **Kristina** for your love and support.

My wonderful family, **Micke, Edvin** and **Axel**, for your love, patience and support. I love you endlessly.

7 REFERENCES

Antoniou AC, Sinilnikova OM, Simard J, Leone M, Dumont M, Neuhausen SL *et al* (2007). RAD51 135G-->C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. *Am J Hum Genet* **81:** 1186-200.

Arias-Lopez C, Lazaro-Trueba I, Kerr P, Lord CJ, Dexter T, Iravani M *et al* (2006). p53 modulates homologous recombination by transcriptional regulation of the RAD51 gene. *EMBO Rep* **7:** 219-24.

Assanah M, Lochhead R, Ogden A, Bruce J, Goldman J, Canoll P (2006). Glial progenitors in adult white matter are driven to form malignant gliomas by platelet-derived growth factor-expressing retroviruses. *J Neurosci* **26:** 6781-90.

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB *et al* (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **444:** 756-60.

Bartek J, Bartkova J, Lukas J (2007a). DNA damage signalling guards against activated oncogenes and tumour progression. *Oncogene* **26:** 7773-9.

Bartek J, Lukas J (2007). DNA damage checkpoints: from initiation to recovery or adaptation. *Curr Opin Cell Biol* **19:** 238-45.

Bartek J, Lukas J, Bartkova J (2007b). DNA damage response as an anti-cancer barrier: damage threshold and the concept of 'conditional haploinsufficiency'. *Cell Cycle* **6:** 2344-7.

Bartkova J, Bakkenist CJ, Rajpert-De Meyts E, Skakkebaek NE, Sehested M, Lukas J *et al* (2005a). ATM activation in normal human tissues and testicular cancer. *Cell Cycle* **4:** 838-45.

Bartkova J, Horejsi Z, Koed K, Kramer A, Tort F, Zieger K *et al* (2005b). DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature* **434:** 864-70.

Bartkova J, Horejsi Z, Sehested M, Nesland JM, Rajpert-De Meyts E, Skakkebaek NE *et al* (2007). DNA damage response mediators MDC1 and 53BP1: constitutive activation and aberrant loss in breast and lung cancer, but not in testicular germ cell tumours. *Oncogene* **26:** 7414-22.

Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N *et al* (2006). Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature* **444:** 633-7.

Baumann P, Benson FE, West SC (1996). Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. *Cell* **87:** 757-66.

Benson FE, Stasiak A, West SC (1994). Purification and characterization of the human Rad51 protein, an analogue of E. coli RecA. *Embo J* **13:** 5764-71.

Bertrand P, Lambert S, Joubert C, Lopez BS (2003). Overexpression of mammalian Rad51 does not stimulate tumorigenesis while a dominant-negative Rad51 affects centrosome fragmentation, ploidy and stimulates tumorigenesis, in p53-defective CHO cells. *Oncogene* **22:** 7587-92.

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E *et al* (2005). Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434:** 913-7.

Brzovic PS, Keeffe JR, Nishikawa H, Miyamoto K, Fox D, 3rd, Fukuda M *et al* (2003). Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. *Proc Natl Acad Sci U S A* **100:** 5646-51.

Carson CT, Schwartz RA, Stracker TH, Lilley CE, Lee DV, Weitzman MD (2003). The Mre11 complex is required for ATM activation and the G2/M checkpoint. *EMBO J* **22:** 6610-20.

Castilla LH, Couch FJ, Erdos MR, Hoskins KF, Calzone K, Garber JE *et al* (1994). Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. *Nat Genet* **8:** 387-91.

Chen A, Kleiman FE, Manley JL, Ouchi T, Pan ZQ (2002). Autoubiquitination of the BRCA1*BARD1 RING ubiquitin ligase. *J Biol Chem* **277:** 22085-92.

Chen G, Yuan SS, Liu W, Xu Y, Trujillo K, Song B *et al* (1999). Radiation-induced assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl. *J Biol Chem* **274:** 12748-52.

Chen J, Silver DP, Walpita D, Cantor SB, Gazdar AF, Tomlinson G *et al* (1998). Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol Cell* **2:** 317-28.

Chi P, Van Komen S, Sehorn MG, Sigurdsson S, Sung P (2006). Roles of ATP binding and ATP hydrolysis in human Rad51 recombinase function. *DNA Repair (Amst)* **5:** 381-91.

Choudhury AD, Xu H, Baer R (2004). Ubiquitination and proteasomal degradation of the BRCA1 tumor suppressor is regulated during cell cycle progression. *J Biol Chem* **279:** 33909-18.

Collins N, McManus R, Wooster R, Mangion J, Seal S, Lakhani SR *et al* (1995). Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13. *Oncogene* **10:** 1673-5.

Collins VP (2004). Brain tumours: classification and genes. *J Neurol Neurosurg Psychiatry* **75 Suppl 2:** ii2-11.

Collis SJ, Tighe A, Scott SD, Roberts SA, Hendry JH, Margison GP (2001). Ribozyme minigene-mediated RAD51 down-regulation increases radiosensitivity of human prostate cancer cells. *Nucleic Acids Res* **29:** 1534-8.

Connell PP, Siddiqui N, Hoffman S, Kuang A, Khatipov EA, Weichselbaum RR *et al* (2004). A hot spot for RAD51C interactions revealed by a peptide that sensitizes cells to cisplatin. *Cancer Res* **64:** 3002-5.

Cornelis RS, Neuhausen SL, Johansson O, Arason A, Kelsell D, Ponder BA *et al* (1995). High allele loss rates at 17q12-q21 in breast and ovarian tumors from BRCAl-linked families. The Breast Cancer Linkage Consortium. *Genes Chromosomes Cancer* **13:** 203-10.

Dai C, Celestino JC, Okada Y, Louis DN, Fuller GN, Holland EC (2001). PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. *Genes Dev* **15:** 1913-25.

Dai C, Lyustikman Y, Shih A, Hu X, Fuller GN, Rosenblum M *et al* (2005). The characteristics of astrocytomas and oligodendrogliomas are caused by two distinct and interchangeable signaling formats. *Neoplasia* **7:** 397-406.

Davies OR, Pellegrini L (2007). Interaction with the BRCA2 C terminus protects RAD51-DNA filaments from disassembly by BRC repeats. *Nat Struct Mol Biol* **14:** 475-83.

Deans B, Griffin CS, Maconochie M, Thacker J (2000). Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. *EMBO J* **19:** 6675-85.

Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C *et al* (2006). Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature* **444:** 638-42.

DiTullio RA, Jr., Mochan TA, Venere M, Bartkova J, Sehested M, Bartek J *et al* (2002). 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer. *Nat Cell Biol* **4:** 998-1002.

Esashi F, Christ N, Gannon J, Liu Y, Hunt T, Jasin M *et al* (2005). CDK-dependent phosphorylation of BRCA2 as a regulatory mechanism for recombinational repair. *Nature* **434:** 598-604.

Esashi F, Galkin VE, Yu X, Egelman EH, West SC (2007). Stabilization of RAD51 nucleoprotein filaments by the C-terminal region of BRCA2. *Nat Struct Mol Biol* **14:** 468-74.

Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V *et al* (2000). Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med* **343:** 1350-4.

Fabbro M, Schuechner S, Au WW, Henderson BR (2004). BARD1 regulates BRCA1 apoptotic function by a mechanism involving nuclear retention. *Exp Cell Res* **298:** 661-73.

Fan C, Quan R, Feng X, Gillis A, He L, Matsumoto ED *et al* (2006). ATM activation is accompanied with earlier stages of prostate tumorigenesis. *Biochim Biophys Acta* **1763:** 1090-7.

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB *et al* (2005). Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434:** 917-21.

Feki A, Jefford CE, Berardi P, Wu JY, Cartier L, Krause KH *et al* (2005). BARD1 induces apoptosis by catalysing phosphorylation of p53 by DNA-damage response kinase. *Oncogene* **24:** 3726-36.

Feki A, Jefford CE, Durand P, Harb J, Lucas H, Krause KH *et al* (2004). BARD1 expression during spermatogenesis is associated with apoptosis and hormonally regulated. *Biol Reprod* **71:** 1614-24.

Flygare J, Benson F, Hellgren D (1996). Expression of the human RAD51 gene during the cell cycle in primary human peripheral blood lymphocytes. *Biochim Biophys Acta* **1312:** 231-6.

Flygare J, Falt S, Ottervald J, Castro J, Dackland AL, Hellgren D *et al* (2001). Effects of HsRad51 overexpression on cell proliferation, cell cycle progression, and apoptosis. *Exp Cell Res* **268:** 61-9.

Fomchenko EI, Holland EC (2005). Stem cells and brain cancer. *Exp Cell Res* **306:** 323-9.

Foray N, Marot D, Randrianarison V, Venezia ND, Picard D, Perricaudet M *et al* (2002). Constitutive association of BRCA1 and c-Abl and its ATM-dependent disruption after irradiation. *Mol Cell Biol* **22:** 4020-32.

Forget AL, Loftus MS, McGrew DA, Bennett BT, Knight KL (2007). The human Rad51 K133A mutant is functional for DNA double-strand break repair in human cells. *Biochemistry* **46:** 3566-75.

Frame FM, Rogoff HA, Pickering MT, Cress WD, Kowalik TF (2006). E2F1 induces MRN foci formation and a cell cycle checkpoint response in human fibroblasts. *Oncogene* **25:** 3258-66.

Francis R, Richardson C (2007). Multipotent hematopoietic cells susceptible to alternative double-strand break repair pathways that promote genome rearrangements. *Genes Dev* **21:** 1064-74.

Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, Rowell SE *et al* (1994). Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nat Genet* **8:** 399-404.

Galkin VE, Esashi F, Yu X, Yang S, West SC, Egelman EH (2005). BRCA2 BRC motifs bind RAD51-DNA filaments. *Proc Natl Acad Sci U S A* **102:** 8537-42.

Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, De Vitis S *et al* (2004). Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* **64:** 7011-21.

Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J *et al* (2001). Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol Cell* **7:** 249-62.

Gerson SL (2004). MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer* **4:** 296-307.

Ghimenti C, Sensi E, Presciuttini S, Brunetti IM, Conte P, Bevilacqua G *et al* (2002). Germline mutations of the BRCA1-associated ring domain (BARD1) gene in breast and breast/ovarian families negative for BRCA1 and BRCA2 alterations. *Genes Chromosomes Cancer* **33:** 235-42.

Golding SE, Rosenberg E, Neill S, Dent P, Povirk LF, Valerie K (2007). Extracellular signal-related kinase positively regulates ataxia telangiectasia mutated, homologous recombination repair, and the DNA damage response. *Cancer Res* **67:** 1046-53.

Golub EI, Kovalenko OV, Gupta RC, Ward DC, Radding CM (1997). Interaction of human recombination proteins Rad51 and Rad54. *Nucleic Acids Res* **25:** 4106-10.

Gonzalez R, Silva JM, Dominguez G, Garcia JM, Martinez G, Vargas J *et al* (1999). Detection of loss of heterozygosity at RAD51, RAD52, RAD54 and BRCA1 and BRCA2 loci in breast cancer: pathological correlations. *Br J Cancer* **81:** 503-9.

Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T *et al* (2005). Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature* **434:** 907-13.

Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH (1996). Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat Genet* **12:** 191-4.

Gupta RC, Bazemore LR, Golub EI, Radding CM (1997). Activities of human recombination protein Rad51. *Proc Natl Acad Sci U S A* **94:** 463-8.

Haaf T, Golub EI, Reddy G, Radding CM, Ward DC (1995). Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes. *Proc Natl Acad Sci U S A* **92:** 2298-302.

Hakem R, de la Pompa JL, Elia A, Potter J, Mak TW (1997). Partial rescue of Brca1 (5-6) early embryonic lethality by p53 or p21 null mutation. *Nat Genet* **16:** 298-302.

Han H, Bearss DJ, Browne LW, Calaluce R, Nagle RB, Von Hoff DD (2002). Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. *Cancer Res* **62:** 2890-6.

Hanahan D, Weinberg RA (2000). The hallmarks of cancer. *Cell* **100:** 57-70.

Hannay JA, Liu J, Zhu QS, Bolshakov SV, Li L, Pisters PW *et al* (2007). Rad51 overexpression contributes to chemoresistance in human soft tissue sarcoma cells: a role for p53/activator protein 2 transcriptional regulation. *Mol Cancer Ther* **6:** 1650-60.

Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y *et al* (2001). The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *J Biol Chem* **276:** 14537-40.

Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M *et al* (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* **352:** 997-1003.

Heldin CH, Westermark B, Wasteson A (1981). Specific receptors for platelet-derived growth factor on cells derived from connective tissue and glia. *Proc Natl Acad Sci U S A* **78:** 3664-8.

Helleday T, Petermann E, Lundin C, Hodgson B, Sharma RA (2008). DNA repair pathways as targets for cancer therapy. *Nat Rev Cancer* **8:** 193-204.

Hellstrom M, Gerhardt H, Kalen M, Li X, Eriksson U, Wolburg H *et al* (2001). Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol* **153:** 543-53.

Hermanson M, Funa K, Hartman M, Claesson-Welsh L, Heldin CH, Westermark B *et al* (1992). Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. *Cancer Res* **52:** 3213-9.

Holland EC (2001). Gliomagenesis: genetic alterations and mouse models. *Nat Rev Genet* **2:** 120-9.

Holland EC, Hively WP, DePinho RA, Varmus HE (1998a). A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. *Genes Dev* **12:** 3675-85.

Holland EC, Hively WP, Gallo V, Varmus HE (1998b). Modeling mutations in the G1 arrest pathway in human gliomas: overexpression of CDK4 but not loss of INK4a-ARF induces hyperploidy in cultured mouse astrocytes. *Genes Dev* **12:** 3644-9.

Holmgren L, Glaser A, Pfeifer-Ohlsson S, Ohlsson R (1991). Angiogenesis during human extraembryonic development involves the spatiotemporal control of PDGF ligand and receptor gene expression. *Development* **113:** 749-54.

Honrado E, Osorio A, Palacios J, Milne RL, Sanchez L, Diez O *et al* (2005). Immunohistochemical expression of DNA repair proteins in familial breast cancer differentiate BRCA2-associated tumors. *J Clin Oncol* **23:** 7503-11.

Huang Y, Nakada S, Ishiko T, Utsugisawa T, Datta R, Kharbanda S *et al* (1999). Role for caspase-mediated cleavage of Rad51 in induction of apoptosis by DNA damage. *Mol Cell Biol* **19:** 2986-97.

Ira G, Pellicioli A, Balijja A, Wang X, Fiorani S, Carotenuto W *et al* (2004). DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature* **431:** 1011-7.

Irminger-Finger I, Leung WC (2002). BRCA1-dependent and independent functions of BARD1. *Int J Biochem Cell Biol* **34:** 582-7.

Irminger-Finger I, Leung WC, Li J, Dubois-Dauphin M, Harb J, Feki A *et al* (2001). Identification of BARD1 as mediator between proapoptotic stress and p53-dependent apoptosis. *Mol Cell* **8:** 1255-66.

Ishitobi M, Miyoshi Y, Hasegawa S, Egawa C, Tamaki Y, Monden M *et al* (2003). Mutational analysis of BARD1 in familial breast cancer patients in Japan. *Cancer Lett* **200:** 1-7.

Ito M, Yamamoto S, Nimura K, Hiraoka K, Tamai K, Kaneda Y (2005). Rad51 siRNA delivered by HVJ envelope vector enhances the anti-cancer effect of cisplatin. *J Gene Med*.

Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, VandenBerg S *et al* (2006). PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron* **51:** 187-99.

Jara L, Acevedo ML, Blanco R, Castro VG, Bravo T, Gomez F *et al* (2007). RAD51 135G>C polymorphism and risk of familial breast cancer in a South American population. *Cancer Genet Cytogenet* **178:** 65-9.

Jasin M (2002). Homologous repair of DNA damage and tumorigenesis: the BRCA connection. *Oncogene* **21:** 8981-93.

Jefford CE, Feki A, Harb J, Krause KH, Irminger-Finger I (2004). Nuclear-cytoplasmic translocation of BARD1 is linked to its apoptotic activity. *Oncogene* **23:** 3509-20.

Jeggo PA (1998). Identification of genes involved in repair of DNA double-strand breaks in mammalian cells. *Radiat Res* **150:** S80-91.

Jin Y, Xu XL, Yang MC, Wei F, Ayi TC, Bowcock AM *et al* (1997). Cell cycle-dependent colocalization of BARD1 and BRCA1 proteins in discrete nuclear domains. *Proc Natl Acad Sci U S A* **94:** 12075-80.

Johansson FK, Brodd J, Eklof C, Ferletta M, Hesselager G, Tiger CF *et al* (2004). Identification of candidate cancer-causing genes in mouse brain tumors by retroviral tagging. *Proc Natl Acad Sci U S A* **101:** 11334-7.

Johansson FK, Goransson H, Westermark B (2005). Expression analysis of genes involved in brain tumor progression driven by retroviral insertional mutagenesis in mice. *Oncogene*.

Joukov V, Chen J, Fox EA, Green JB, Livingston DM (2001). Functional communication between endogenous BRCA1 and its partner, BARD1, during Xenopus laevis development. *Proc Natl Acad Sci U S A* **98:** 12078-83.

Kadouri L, Kote-Jarai Z, Hubert A, Durocher F, Abeliovich D, Glaser B *et al* (2004). A single-nucleotide polymorphism in the RAD51 gene modifies breast cancer risk in BRCA2 carriers, but not in BRCA1 carriers or noncarriers. *Br J Cancer* **90:** 2002-5.

Kaminski WE, Lindahl P, Lin NL, Broudy VC, Crosby JR, Hellstrom M *et al* (2001). Basis of hematopoietic defects in platelet-derived growth factor (PDGF)-B and PDGF beta-receptor null mice. *Blood* **97:** 1990-8.

Karppinen SM, Barkardottir RB, Backenhorn K, Sydenham T, Syrjakoski K, Schleutker J *et al* (2006). Nordic collaborative study of the BARD1 Cys557Ser allele in 3956 patients with cancer: enrichment in familial BRCA1/BRCA2 mutation-negative breast cancer but not in other malignancies. *J Med Genet* **43:** 856-62.

Karppinen SM, Heikkinen K, Rapakko K, Winqvist R (2004). Mutation screening of the BARD1 gene: evidence for involvement of the Cys557Ser allele in hereditary susceptibility to breast cancer. *J Med Genet* **41:** e114.

Kato M, Yano K, Matsuo F, Saito H, Katagiri T, Kurumizaka H *et al* (2000). Identification of Rad51 alteration in patients with bilateral breast cancer. *J Hum Genet* **45:** 133-7.

Kim H, Chen J, Yu X (2007). Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science* **316:** 1202-5.

Kim HS, Li H, Cevher M, Parmelee A, Fonseca D, Kleiman FE *et al* (2006). DNA damage-induced BARD1 phosphorylation is critical for the inhibition of messenger RNA processing by BRCA1/BARD1 complex. *Cancer Res* **66:** 4561-5.

Kleiman FE, Manley JL (2001). The BARD1-CstF-50 interaction links mRNA 3' end formation to DNA damage and tumor suppression. *Cell* **104:** 743-53.

Kleiman FE, Wu-Baer F, Fonseca D, Kaneko S, Baer R, Manley JL (2005). BRCA1/BARD1 inhibition of mRNA 3' processing involves targeted degradation of RNA polymerase II. *Genes Dev* **19:** 1227-37.

Laufer M, Nandula SV, Modi AP, Wang S, Jasin M, Murty VV *et al* (2007). Structural requirements for the BARD1 tumor suppressor in chromosomal stability and homology-directed DNA repair. *J Biol Chem* **282:** 34325-33.

Lengauer C, Kinzler KW, Vogelstein B (1998). Genetic instabilities in human cancers. *Nature* **396:** 643-9.

Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C (1994). Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev* **8:** 1875-87.

Levy-Lahad E, Lahad A, Eisenberg S, Dagan E, Paperna T, Kasinetz L *et al* (2001). A single nucleotide polymorphism in the RAD51 gene modifies cancer risk in BRCA2 but not BRCA1 carriers. *Proc Natl Acad Sci U S A* **98:** 3232-6.

Li L, Ryser S, Dizin E, Pils D, Krainer M, Jefford CE *et al* (2007). Oncogenic BARD1 isoforms expressed in gynecological cancers. *Cancer Res* **67:** 11876-85.

Lim DS, Hasty P (1996). A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol Cell Biol* **16:** 7133-43.

Lindahl P, Johansson BR, Leveen P, Betsholtz C (1997). Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* **277:** 242-5.

Linke SP, Sengupta S, Khabie N, Jeffries BA, Buchhop S, Miska S *et al* (2003). p53 interacts with hRAD51 and hRAD54, and directly modulates homologous recombination. *Cancer Res* **63:** 2596-605.

Liu CY, Flesken-Nikitin A, Li S, Zeng Y, Lee WH (1996). Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev* **10:** 1835-43.

Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM (1999). RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc Natl Acad Sci U S A* **96:** 11364-9.

Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A (1997). Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev* **11:** 1226-41.

Lundin C, Schultz N, Arnaudeau C, Mohindra A, Hansen LT, Helleday T (2003). RAD51 is involved in repair of damage associated with DNA replication in mammalian cells. *J Mol Biol* **328:** 521-35.

Maacke H, Jost K, Opitz S, Miska S, Yuan Y, Hasselbach L *et al* (2000a). DNA repair and recombination factor Rad51 is over-expressed in human pancreatic adenocarcinoma. *Oncogene* **19:** 2791-5.

Maacke H, Opitz S, Jost K, Hamdorf W, Henning W, Kruger S *et al* (2000b). Over-expression of wild-type Rad51 correlates with histological grading of invasive ductal breast cancer. *Int J Cancer* **88:** 907-13.

Mallery DL, Vandenberg CJ, Hiom K (2002). Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. *EMBO J* **21:** 6755-62.

Mallette FA, Gaumont-Leclerc MF, Ferbeyre G (2007). The DNA damage signaling pathway is a critical mediator of oncogene-induced senescence. *Genes Dev* **21:** 43-8.

Martin RW, Orelli BJ, Yamazoe M, Minn AJ, Takeda S, Bishop DK (2007). RAD51 up-regulation bypasses BRCA1 function and is a common feature of BRCA1-deficient breast tumors. *Cancer Res* **67:** 9658-65.

Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ *et al* (2001). Identification and purification of two distinct complexes containing the five RAD51 paralogs. *Genes Dev* **15:** 3296-307.

McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S *et al* (2006). Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res* **66:** 8109-15.

McCarthy EE, Celebi JT, Baer R, Ludwig T (2003). Loss of Bard1, the heterodimeric partner of the Brca1 tumor suppressor, results in early embryonic lethality and chromosomal instability. *Mol Cell Biol* **23:** 5056-63.

Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A (2006). Origin of oligodendrocytes in the subventricular zone of the adult brain. *J Neurosci* **26:** 7907-18.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S *et al* (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266:** 66-71.

Morris JR, Solomon E (2004). BRCA1 : BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Hum Mol Genet* **13:** 807-17.

Morrison C, Shinohara A, Sonoda E, Yamaguchi-Iwai Y, Takata M, Weichselbaum RR *et al* (1999). The essential functions of human Rad51 are independent of ATP hydrolysis. *Mol Cell Biol* **19:** 6891-7.

Moynahan ME, Chiu JW, Koller BH, Jasin M (1999). Brca1 controls homology-directed DNA repair. *Mol Cell* **4:** 511-8.

Moynahan ME, Cui TY, Jasin M (2001a). Homology-directed dna repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation. *Cancer Res* **61:** 4842-50.

Moynahan ME, Pierce AJ, Jasin M (2001b). BRCA2 is required for homology-directed repair of chromosomal breaks. *Mol Cell* **7:** 263-72.

Nowacka-Zawisza M, Brys M, Romanowicz-Makowska H, Kulig A, Krajewska WM (2007). Genetic instability in the RAD51 and BRCA1 regions in breast cancer. *Cell Mol Biol Lett* **12:** 192-205.

Nuciforo PG, Luise C, Capra M, Pelosi G, d'Adda di Fagagna F (2007). Complex engagement of DNA damage response pathways in human cancer and in lung tumor progression. *Carcinogenesis* **28:** 2082-8.

Ohgaki H, Kleihues P (2005). Epidemiology and etiology of gliomas. *Acta Neuropathol* **109:** 93-108.

Ohnishi T, Taki T, Hiraga S, Arita N, Morita T (1998). In vitro and in vivo potentiation of radiosensitivity of malignant gliomas by antisense inhibition of the RAD51 gene. *Biochem Biophys Res Commun* **245:** 319-24.

Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM (2000). A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr Biol* **10:** 886-95.

Petalcorin MI, Galkin VE, Yu X, Egelman EH, Boulton SJ (2007). Stabilization of RAD-51-DNA filaments via an interaction domain in Caenorhabditis elegans BRCA2. *Proc Natl Acad Sci U S A* **104:** 8299-304.

Pickering MT, Kowalik TF (2006). Rb inactivation leads to E2F1-mediated DNA double-strand break accumulation. *Oncogene* **25:** 746-55.

Pierce AJ, Stark JM, Araujo FD, Moynahan ME, Berwick M, Jasin M (2001). Double-strand breaks and tumorigenesis. *Trends Cell Biol* **11:** S52-9.

Pittman DL, Schimenti JC (2000). Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3. *Genesis* **26:** 167-73.

Powers JT, Hong S, Mayhew CN, Rogers PM, Knudsen ES, Johnson DG (2004). E2F1 uses the ATM signaling pathway to induce p53 and Chk2 phosphorylation and apoptosis. *Mol Cancer Res* **2:** 203-14.

Pusapati RV, Rounbehler RJ, Hong S, Powers JT, Yan M, Kiguchi K *et al* (2006). ATM promotes apoptosis and suppresses tumorigenesis in response to Myc. *Proc Natl Acad Sci U S A* **103:** 1446-51.

Raderschall E, Bazarov A, Cao J, Lurz R, Smith A, Mann W *et al* (2002a). Formation of higher-order nuclear Rad51 structures is functionally linked to p21 expression and protection from DNA damage-induced apoptosis. *J Cell Sci* **115:** 153-64.

Raderschall E, Golub EI, Haaf T (1999). Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. *Proc Natl Acad Sci U S A* **96:** 1921-6.

Raderschall E, Stout K, Freier S, Suckow V, Schweiger S, Haaf T (2002b). Elevated levels of Rad51 recombination protein in tumor cells. *Cancer Res* **62:** 219-25.

Reimann M, Loddenkemper C, Rudolph C, Schildhauer I, Teichmann B, Stein H *et al* (2007). The Myc-evoked DNA damage response accounts for treatment resistance in primary lymphomas in vivo. *Blood* **110:** 2996-3004.

Rich JN, Bigner DD (2004). Development of novel targeted therapies in the treatment of malignant glioma. *Nat Rev Drug Discov* **3:** 430-46.

Richardson C, Stark JM, Ommundsen M, Jasin M (2004). Rad51 overexpression promotes alternative double-strand break repair pathways and genome instability. *Oncogene* **23:** 546-53.

Rodriguez JA, Schuchner S, Au WW, Fabbro M, Henderson BR (2004). Nuclear-cytoplasmic shuttling of BARD1 contributes to its proapoptotic activity and is regulated by dimerization with BRCA1. *Oncogene* **23:** 1809-20.

Ross R, Glomset J, Kariya B, Harker L (1974). A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. *Proc Natl Acad Sci U S A* **71:** 1207-10.

Ruffner H, Joazeiro CA, Hemmati D, Hunter T, Verma IM (2001). Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc Natl Acad Sci U S A* **98:** 5134-9.

Russell JS, Brady K, Burgan WE, Cerra MA, Oswald KA, Camphausen K *et al* (2003). Gleevec-mediated inhibition of Rad51 expression and enhancement of tumor cell radiosensitivity. *Cancer Res* **63:** 7377-83.

Sanson M, Thillet J, Hoang-Xuan K (2004). Molecular changes in gliomas. *Curr Opin Oncol* **16:** 607-13.

Sauer MK, Andrulis IL (2005). Identification and characterization of missense alterations in the BRCA1 associated RING domain (BARD1) gene in breast and ovarian cancer. *J Med Genet* **42:** 633-8.

Saydam O, Saydam N, Glauser DL, Pruschy M, Dinh-Van V, Hilbe M *et al* (2007). HSV-1 amplicon-mediated post-transcriptional inhibition of Rad51 sensitizes human glioma cells to ionizing radiation. *Gene Ther* **14:** 1143-51.

Schultz LB, Chehab NH, Malikzay A, Halazonetis TD (2000). p53 binding protein 1 (53BP1) is an early participant in the cellular response to DNA double-strand breaks. *J Cell Biol* **151:** 1381-90.

Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J *et al* (1997). Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell* **88:** 265-75.

Scully R, Puget N, Vlasakova K (2000). DNA polymerase stalling, sister chromatid recombination and the BRCA genes. *Oncogene* **19:** 6176-83.

Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C *et al* (1997). Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* **386:** 804-10.

Shih AH, Dai C, Hu X, Rosenblum MK, Koutcher JA, Holland EC (2004). Dose-dependent effects of platelet-derived growth factor-B on glial tumorigenesis. *Cancer Res* **64:** 4783-9.

Shih AH, Holland EC (2006). Platelet-derived growth factor (PDGF) and glial tumorigenesis. *Cancer Lett* **232:** 139-47.

Short SC, Martindale C, Bourne S, Brand G, Woodcock M, Johnston P (2007). DNA repair after irradiation in glioma cells and normal human astrocytes. *Neuro Oncol* **9:** 404-11.

Silva RL, Thornton JD, Martin AC, Rehg JE, Bertwistle D, Zindy F *et al* (2005). Arf-dependent regulation of Pdgf signaling in perivascular cells in the developing mouse eye. *EMBO J* **24:** 2803-14.

Simons AM, Horwitz AA, Starita LM, Griffin K, Williams RS, Glover JN *et al* (2006). BRCA1 DNA-binding activity is stimulated by BARD1. *Cancer Res* **66:** 2012-8.

Sims DE (1986). The pericyte--a review. *Tissue Cell* **18:** 153-74.

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J *et al* (2003). Identification of a cancer stem cell in human brain tumors. *Cancer Res* **63:** 5821-8.

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T *et al* (2004). Identification of human brain tumour initiating cells. *Nature* **432:** 396-401.

Sleeth KM, Sorensen CS, Issaeva N, Dziegielewski J, Bartek J, Helleday T (2007). RPA mediates recombination repair during replication stress and is displaced from DNA by checkpoint signalling in human cells. *J Mol Biol* **373:** 38-47.

Slupianek A, Hoser G, Majsterek I, Bronisz A, Malecki M, Blasiak J *et al* (2002). Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G(2)/M phase, and protection from apoptosis. *Mol Cell Biol* **22:** 4189-201.

Slupianek A, Schmutte C, Tombline G, Nieborowska-Skorska M, Hoser G, Nowicki MO *et al* (2001). BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. *Mol Cell* **8:** 795-806.

Smiraldo PG, Gruver AM, Osborn JC, Pittman DL (2005). Extensive chromosomal instability in Rad51d-deficient mouse cells. *Cancer Res* **65:** 2089-96.

Snouwaert JN, Gowen LC, Lee V, Koller BH (1998). Characterization of Brca1 deficient mice. *Breast Dis* **10:** 33-44.

Sobhian B, Shao G, Lilli DR, Culhane AC, Moreau LA, Xia B *et al* (2007). RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* **316:** 1198-202.

Sonoda E, Sasaki MS, Buerstedde JM, Bezzubova O, Shinohara A, Ogawa H *et al* (1998). Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *Embo J* **17:** 598-608.

Stacey SN, Sulem P, Johannsson OT, Helgason A, Gudmundsson J, Kostic JP *et al* (2006). The BARD1 Cys557Ser variant and breast cancer risk in Iceland. *PLoS Med* **3:** e217.

Starita LM, Horwitz AA, Keogh MC, Ishioka C, Parvin JD, Chiba N (2005). BRCA1/BARD1 ubiquitinate phosphorylated RNA polymerase II. *J Biol Chem* **280:** 24498-505.

Starita LM, Machida Y, Sankaran S, Elias JE, Griffin K, Schlegel BP *et al* (2004). BRCA1-dependent ubiquitination of gamma-tubulin regulates centrosome number. *Mol Cell Biol* **24:** 8457-66.

Stark JM, Hu P, Pierce AJ, Moynahan ME, Ellis N, Jasin M (2002). ATP hydrolysis by mammalian RAD51 has a key role during homology-directed DNA repair. *J Biol Chem* **277:** 20185-94.

Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ *et al* (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* **352:** 987-96.

Sturzbecher HW, Donzelmann B, Henning W, Knippschild U, Buchhop S (1996). p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. *Embo J* **15:** 1992-2002.

Taki T, Ohnishi T, Yamamoto A, Hiraga S, Arita N, Izumoto S *et al* (1996). Antisense inhibition of the RAD51 enhances radiosensitivity. *Biochem Biophys Res Commun* **223:** 434-8.

Tan TL, Essers J, Citterio E, Swagemakers SM, de Wit J, Benson FE *et al* (1999). Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation. *Curr Biol* **9:** 325-8.

Tchougounova E, Kastemar M, Brasater D, Holland EC, Westermark B, Uhrbom L (2007). Loss of Arf causes tumor progression of PDGFB-induced oligodendroglioma. *Oncogene* **26:** 6289-96.

Tembe V, Henderson BR (2007). BARD1 translocation to mitochondria correlates with Bax oligomerization, loss of mitochondrial membrane potential, and apoptosis. *J Biol Chem* **282:** 20513-22.

Thai TH, Du F, Tsan JT, Jin Y, Phung A, Spillman MA *et al* (1998). Mutations in the BRCA1-associated RING domain (BARD1) gene in primary breast, ovarian and uterine cancers. *Hum Mol Genet* **7:** 195-202.

Thompson LH, Schild D (1999). The contribution of homologous recombination in preserving genome integrity in mammalian cells. *Biochimie* **81:** 87-105.

Thornton JD, Silva RL, Martin AC, Skapek SX (2005). The Arf tumor suppressor regulates platelet-derived growth factor receptor beta signaling: a new view through the eyes of Arf(-/-) mice. *Cell Cycle* **4:** 1316-9.

Tort F, Bartkova J, Sehested M, Orntoft T, Lukas J, Bartek J (2006). Retinoblastoma pathway defects show differential ability to activate the constitutive DNA damage response in human tumorigenesis. *Cancer Res* **66:** 10258-63.

Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M *et al* (1996). Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc Natl Acad Sci U S A* **93:** 6236-40.

Uhrbom L, Dai C, Celestino JC, Rosenblum MK, Fuller GN, Holland EC (2002). Ink4a-Arf loss cooperates with KRas activation in astrocytes and neural progenitors to generate glioblastomas of various morphologies depending on activated Akt. *Cancer Res* **62:** 5551-8.

Uhrbom L, Hesselager G, Nister M, Westermark B (1998). Induction of brain tumors in mice using a recombinant platelet-derived growth factor B-chain retrovirus. *Cancer Res* **58:** 5275-9.

Uhrbom L, Hesselager G, Ostman A, Nister M, Westermark B (2000). Dependence of autocrine growth factor stimulation in platelet-derived growth factor-B-induced mouse brain tumor cells. *Int J Cancer* **85:** 398-406.

Uhrbom L, Holland EC (2001). Modeling gliomagenesis with somatic cell gene transfer using retroviral vectors. *J Neurooncol* **53:** 297-305.

Uhrbom L, Kastemar M, Johansson FK, Westermark B, Holland EC (2005). Cell type-specific tumor suppression by ink4a and arf in kras-induced mouse gliomagenesis. *Cancer Res* **65:** 2065-9.

Uhrbom L, Nerio E, Holland EC (2004). Dissecting tumor maintenance requirements using bioluminescence imaging of cell proliferation in a mouse glioma model. *Nat Med* **10:** 1257-60.

Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A, Gygi SP *et al* (2007). Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science* **316:** 1194-8.

Welcsh PL, King MC (2001). BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Hum Mol Genet* **10:** 705-13.

Westermark B, Heldin CH, Nister M (1995). Platelet-derived growth factor in human glioma. *Glia* **15:** 257-63.

Westermark UK, Forsberg N, Bråsäter D, Helgadottir HR, Eriksson A, Zetterberg A *et al* (2008). RAD51 suppresses PDGFB-induced gliomagenesis and genetic instability in wild type and Arf-/- mice. *Submitted*.

Westermark UK, Reyngold M, Olshen AB, Baer R, Jasin M, Moynahan ME (2003). BARD1 participates with BRCA1 in homology-directed repair of chromosome breaks. *Mol Cell Biol* **23:** 7926-36.

Vispe S, Cazaux C, Lesca C, Defais M (1998). Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. *Nucleic Acids Res* **26:** 2859-64.

Wu JY, Vlastos AT, Pelte MF, Caligo MA, Bianco A, Krause KH *et al* (2006). Aberrant expression of BARD1 in breast and ovarian cancers with poor prognosis. *Int J Cancer* **118:** 1215-26.

Wu L, Davies SL, Levitt NC, Hickson ID (2001). Potential role for the BLM helicase in recombinational repair via a conserved interaction with RAD51. *J Biol Chem* **276:** 19375-81.

Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL *et al* (1996). Identification of a RING protein that can interact in vivo with the BRCA1 gene product. *Nat Genet* **14:** 430-40.

Wu-Baer F, Lagrazon K, Yuan W, Baer R (2003). The BRCA1/BARD1 heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue K6 of ubiquitin. *J Biol Chem* **278:** 34743-6.

Xia SJ, Shammas MA, Shmookler Reis RJ (1997). Elevated recombination in immortal human cells is mediated by HsRAD51 recombinase. *Mol Cell Biol* **17:** 7151-8.

Xia Y, Pao GM, Chen HW, Verma IM, Hunter T (2003). Enhancement of BRCA1 E3 ubiquitin ligase activity through direct interaction with the BARD1 protein. *J Biol Chem* **278:** 5255-63.

Yanagisawa T, Urade M, Yamamoto Y, Furuyama J (1998). Increased expression of human DNA repair genes, XRCC1, XRCC3 and RAD51, in radioresistant human KB carcinoma cell line N10. *Oral Oncol* **34:** 524-8.

Yang H, Li Q, Fan J, Holloman WK, Pavletich NP (2005). The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA-ssDNA junction. *Nature* **433:** 653-7.

Yu J, Ustach C, Kim HR (2003). Platelet-derived growth factor signaling and human cancer. *J Biochem Mol Biol* **36:** 49-59.

Yu X, Baer R (2000). Nuclear localization and cell cycle-specific expression of CtIP, a protein that associates with the BRCA1 tumor suppressor. *J Biol Chem* **275:** 18541-9.

Yuan ZM, Huang Y, Ishiko T, Nakada S, Utsugisawa T, Kharbanda S *et al* (1998). Regulation of Rad51 function by c-Abl in response to DNA damage. *J Biol Chem* **273:** 3799-802.