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Role of RAD51AP1 in homologous recombination DNA repair and carcinogenesis

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Abstract

Homologous recombination (HR) serves to repair DNA double-strand breaks and damaged replication forks and is essential for maintaining genome stability and tumor suppression. HR capacity also determines the efficacy of anticancer therapy. Hence, there is an urgent need to better understand all HR proteins and sub-pathways. An emerging protein that is critical for RAD51-mediated HR is RAD51-associated protein 1 (RAD51AP1). Although much has been learned about its biochemical attributes, the precise molecular role of RAD51AP1 in the HR reaction is not yet fully understood. The available literature also suggests that RAD51AP1 expression may be relevant for cancer development and progression. Here, we review the efforts that led to the discovery of RAD51AP1 and elaborate on our current understanding of its biochemical profile and biological function. We also discuss how RAD51AP1 may help to promote cancer development and why it could potentially represent a promising new target for therapeutic intervention.

Graphical abstract

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Conflict of interest statement

The authors declare that there is no conflict of interest.

1. Introduction

The root cause of cancer and many other diseases is DNA mutation and loss of genome integrity. Both can result from DNA lesions of which the DNA double-strand break (DSB) is considered the most deleterious. DSBs are induced exogenously from *e.g.* exposure to ionizing radiation (IR) or by endogenous sources including reactive oxygen species (ROS) generated during cellular metabolism [1]. Cells respond to DSBs via DNA damage response pathways that activate DNA damage checkpoints and DNA damage repair mechanisms. One such pathway for mending DSBs efficiently is homologous recombination DNA repair (HR), a highly conserved process critical for tumor suppression. In the HR reaction, a homologous template DNA sequence is engaged, allowing for the faithful restoration of lost sequence information at the break site [2, 3].

HR is catalyzed by the RAD51 recombinase [4], which forms a nucleoprotein filament on resected single-stranded DNA (ssDNA) at the damage site. The RAD51-ssDNA nucleoprotein filament, also called the presynaptic filament, captures duplex DNA and generates a displacement loop (D-loop) upon location of a homologous DNA target. Subsequent DNA synthesis and resolution completes HR DNA repair [5]. The repair process requires many mediator proteins that ensure the efficiency of RAD51-ssDNA filament assembly and filament stability; key HR mediators include the human breast cancer susceptibility gene products BRCA1, BRCA2, and PALB2, and the acidic protein DSS1 [6–13]. Emerging evidence suggests that these HR mediators may also enhance the activity of the RAD51-related meiotic recombinase DMC1.

Besides the aforementioned HR proteins, a key protein that is critical for HR downstream of RAD51 filament formation is RAD51-associated protein 1 (RAD51AP1) [7, 14–16]. Much has been learned about the biochemical attributes of this RAD51- and DMC1-interacting protein since its discovery in 1997 [7, 14]. However, much remains to be learned about the molecular role of RAD51AP1 in the HR reaction and its impact on cancer initiation and progression remains to be elucidated. Here, we revisit the discovery of RAD51AP1 and review the current understanding of the biochemical and biological functions of this HR factor. We also discuss *our* view regarding how RAD51AP1 may help to promote cancer development and potentially be a promising new target for therapeutic intervention.

2. Attributes of the RAD51AP1 protein

2.1. Discovery of RAD51AP1 as a RAD51-interacting protein

RAD51AP1 was discovered in 1997 as a RAD51-interacting protein using the yeast two-hybrid (Y2H) system and was originally termed Pir51 and RAB22 for the human and mouse proteins, respectively [7, 14]. In the Y2H system and in co-immunoprecipitation experiments, RAD51AP1 was shown to interact with RAD51 with high affinity [7, 14, 17]. Follow-up work led to mapping of the RAD51-binding domain onto the C-terminal 26 residues of RAD51AP1 [15, 16, 18]. Consistent with its role in HR DNA repair, RAD51AP1 foci were shown to co-localize with RAD51 foci spontaneously and after DNA damage induction in CHO-K1 and human cells [7, 15, 16, 19].

The human *RAD51AP1* gene was mapped to chromosome 12p13.1–13.2 [14], a region that shows homology with the distal portion of mouse chromosome 6 and that includes the mouse *Rad51ap1* gene [7]. *RAD51AP1* is highly conserved among vertebrates [20]. However, *RAD51AP1* orthologs also exist in a number of invertebrates, but are absent in model organisms including *D. melanogaster*, *C. elegans*, and *S. cerevisiae* [20].

The tissue-specific distribution of the human *RAD51AP1* transcript is similar to that of *RAD51*, *BRCA1*, and *BRCA2*, being the highest in thymus, bone marrow, spleen, and testis [19], which correlates well with what has been observed in mice [7]. This expression pattern is consistent with a key role of *RAD51AP1* and HR in the mitigation of spontaneous DNA damage in proliferating cells. Of the four *RAD51AP1* splice variants, two have been shown to code for a functional protein (<http://www.proteinatlas.org/>). Isoform 1 and isoform 2 are 352 and 335 amino acids in length, respectively, and hydrophilic with a third of their overall composition as aspartic acid, arginine, glutamic acid, and lysine residues [14, 20]. Both isoforms behave similarly in cell-based and biochemical assays designed to assess HR capacity [15, 16, 21–23].

2.2. DNA-binding and HR activity of *RAD51AP1*

RAD51AP1 binds ssDNA and double-stranded DNA (dsDNA), with a preference for the latter species [14–16]. However, *RAD51AP1* has the highest affinity for branched DNA substrates and the D-loop, indicative of a role in the formation of DNA intermediates in the HR reaction [15, 16, 20, 22, 24, 25]. Via its DNA binding attribute, *RAD51AP1* stimulates the *RAD51*-mediated D-loop reaction in amounts sub-stoichiometric to that of *RAD51* [15, 16]. *RAD51AP1* possesses two distinct DNA-binding domains, with one being located within the N-terminal region and the other residing near the C-terminus of the protein [16, 22]. Both domains are required for the full activity of *RAD51AP1* in protecting cells from DNA damaging agents and in promoting D-loop formation by *RAD51* [22].

In the D-loop reaction, the *RAD51* presynaptic filament engages with a dsDNA partner molecule to conduct homology search and synaptic complex formation, in which homologous DNA sequences are aligned to allow for base switching [26, 27]. Interestingly, C-terminally truncated *RAD51AP1* (C26) and variants of *RAD51AP1* with mutations within the last 26 residues are impaired for *RAD51* interaction and for the ability to stimulate D-loop formation [15, 16, 18]. We surmise that *RAD51AP1* bridges the incoming duplex DNA with the presynaptic filament (Fig. 1A), and it is interesting to note that human *RAD51AP1* has an elongated configuration, as determined by sedimentation equilibrium and electrospray experiments [16]. More mechanistic work will be needed to delineate the spatial and temporal organization of *RAD51AP1* in relation to the presynaptic filament during the duplex engagement, homology search and joint molecule formation steps in the HR reaction.

2.3. *RAD51AP1*-interacting proteins

PALB2 (Partner and Localizer of *BRCA2*), a tumor suppressor that assists the recombination mediator *BRCA2* in the presynaptic stage of HR [13], interacts with the N-terminal region of *RAD51AP1* (Fig. 1B). PALB2 functions cooperatively with *RAD51AP1* in enhancing the

recombinase function of RAD51 in the D-loop reaction and during synaptic complex formation [25]. In spite of the fact that PALB2 and RAD51AP1 can individually promote RAD51-mediated DNA strand invasion, their synergy in this reaction speaks to the intricate crosstalk among the proteins that function in this highly regulated step of HR [18]. Although PALB2-deficient human fibroblasts express RAD51AP1 protein, they cannot assemble RAD51AP1 foci upon treatment with the DNA crosslinking agent mitomycin C (MMC) [25], in support of the key role of PALB2 in the recruitment of RAD51AP1 to DNA damage.

In a large-scale proteomic screen [28], UAF1-USP1, a complex responsible for the deubiquitylation of mono-ubiquitylated FANCD2 [29, 30], was found to associate with RAD51AP1. A small ubiquitin-like modifier (SUMO)-interacting motif (SIM) in RAD51AP1 (Fig. 1B) was identified and shown to associate with two SUMO-like domains (SLDs) in UAF1 [31]. RAD51AP1 and UAF1 act synergistically in RAD51-mediated synaptic complex and D-loop formation assays [31]. Together, these results have uncovered a bridging function for RAD51AP1 between UAF1 and RAD51 at the synaptic stage of HR, and suggest the possibility that RAD51AP1 may represent yet another Fanconi Anemia (FA) gene [31] that could function downstream [15] of FANCD2 mono-ubiquitylation. Both USP1 and UAF1 affect the intracellular stability of RAD51AP1, which appears to be degraded by the proteasome [32]. Cells expressing mutant versions of RAD51AP1 or UAF1 that are compromised for complex formation are hypersensitive to various genotoxic agents including MMC, the topoisomerase I inhibitor camptothecin, and the PARP inhibitor olaparib, and are also impaired for HR [31, 32] highlighting the importance of the RAD51AP1-UAF1 complex in HR-mediated DNA damage repair. GFP-tagged RAD51AP1 was shown to interact with DNA Ligase 3, PARP1, and the topoisomerases TOP2A and TOP2B in a recent proteomic analysis [33]. However, the significance of these interactions remains to be elucidated.

Interestingly, RAD51AP1 also interacts with the meiotic recombinase DMC1 and, similar to its activating ability on RAD51, exerts stimulatory effects on DMC1-mediated duplex DNA engagement, synaptic complex assembly, and D-loop formation [21]. As with RAD51AP1 and RAD51 [15, 16], the stimulatory effect of RAD51AP1 on DMC1 is dependent of their physical interaction. In support of the biochemical data, RAD51AP1 colocalizes with a subset of DMC1 foci on meiotic chromatin in mouse spermatocytes [21]. The DMC1-RAD51AP1 interaction is facilitated by a C-terminal, conserved peptide motif in RAD51AP1 (WVPP), reminiscent of the DMC1-interacting FVPP motif in BRCA2 [34], which lies directly downstream of RAD51AP1's C-terminal DNA binding region and does not overlap with its RAD51 interaction domain [21–23] (Fig. 1B). Consequently, mutant versions of RAD51AP1 that have lost their ability to interact with RAD51 are proficient for DMC1 association [21], supporting the notion that in addition to its role in mitotic DSB repair, RAD51AP1 also serves a distinct function in meiotic HR.

2.4. Homology between RAD51AP1 and other proteins

RAD51AP1 shares extensive (28% identical and 11% similar) sequence homology with the NUCKS1 (Nuclear Casein Kinase and Cyclin-dependent Kinase Substrate 1) protein, indicating that these proteins are paralogs [24]. NUCKS1, however, does not harbor any

sequence that resembles the RAD51AP1 C-terminal RAD51 interaction domain. Consistent with this observation, NUCKS1 and RAD51 do not physically interact [24].

The vertebrate-specific RAD51-Associated Protein 2 (RAD51AP2), which is expressed in meiotic tissues only, shares the conserved RAD51 interaction domain found in RAD51AP1 [18]. However, aside from the RAD51 interaction domain, RAD51AP1 and RAD51AP2 share no further regions of homology [18].

2.5. RAD51AP1 protects against DNA damage

In mitotic cells, RAD51AP1 activates the RAD51 recombinase and promotes the repair of DSBs by HR [14–16]. Knockdown of RAD51AP1 in human cells or deletion of *Rad51ap1* in chicken DT40 cells increases their sensitivity to the cytotoxic effects of DNA cross-linking agents and to IR [15–17, 20]. Knockdown of RAD51AP1 also leads to increased levels of genome instability and decreased homology-directed repair [15, 16]. Taken together, these findings support the important role of RAD51AP1 in HR DNA repair and in maintaining genome stability.

Several studies investigated if RAD51AP1 expression is altered in response to DNA damaging agents or cell cycle stage. In wild type TP53 HCT116 cells, the level of RAD51AP1 remains unchanged upon exposure to doxorubicin, UV light, or γ -rays [17]. The RAD51AP1 level in HeLa cells is also unresponsive to UV light or MMC treatment. However, RAD51AP1 protein can be induced by hydroxyurea (HU) in HeLa cells [17]. *Rad51ap1* transcript increases in a dose-dependent manner in mouse lymphoma cells after HU treatment [35], and in human melanocytes following γ -ray exposure [36]. In the EKT-1 cholangiocarcinoma cells, RAD51AP1 protein level is significantly upregulated at 12 and 24 h after exposure to γ -rays [19]. Similarly, the RAD51AP1 amount increases in HCT116 cells at 12 and 24 h post γ -irradiation and returns to basal levels at 48 h post exposure [19]. In G₀/G₁ T24 bladder carcinoma cells, *RAD51AP1* transcript is essentially undetectable but increases and remains at a high level throughout S, G₂ and M phase [17]. The level of RAD51AP1 protein follows the same expression pattern [17]. Correspondingly, *RAD51AP1* has been picked up in a screen to identify new E2F target genes important for cell proliferation in response to growth stimulation [37]. Taken together, these results show that RAD51AP1 protein and transcript are regulated in a cell cycle-specific manner, being expressed at higher levels in proliferating cells, and are induced upon exposure to HU or IR.

2.6. RAD51AP1 maintains replication fork stability

The protection of DNA replication forks and the repair of damaged replication forks are essential for genome stability and cancer avoidance, and the slowing of replication fork progression, commonly referred to as replication stress, has emerged as a significant source of genome instability during early stages of carcinogenesis [38, 39]. Besides ROS-induced DNA-damage, replication stress can result from a variety of other sources, such as nucleotide depletion, conflicts between DNA replication and transcription, and oncogene activation leading to accelerated cell division. Hormonal signaling and inflammatory processes may exert a similar effect as oncogene activation by coercing cells to undergo high levels of transcription [40].

As demonstrated in DNA combing experiments, loss of RAD51AP1 leads to slowing of DNA replication fork progression and elevated DNA replication stress [20, 24]. To resolve these replication abnormalities, *RAD51AP1*-deficient cells increase their usage of replication origins whereby the timely completion of DNA replication is ensured and further replication stress is minimized [20, 24]. These results show that RAD51AP1 is required for replication fork stability and the avoidance of replication stress. These results also may explain why *RAD51AP1* transcript and protein increase upon exposure of cells to HU and IR [17, 19, 35, 36, 41].

The cellular response to replication stress is mediated by the ataxia telangiectasia and Rad3-related kinase (ATR) which activates the intra S-phase checkpoint [42]. In a quantitative proteomic study to define cellular signaling after replication stress, RAD51AP1 was discovered as a putative target of ATR [33]. RAD51AP1 S120 (in isoform 1; Fig. 2) is phosphorylated by ATR upon nucleotide depletion [33], providing more evidence for a role of RAD51AP1 in the replication stress response. To gain further insights into the function of RAD51AP1 during replication stress, Wagner *et al.* [33] also analyzed the interaction partners of RAD51AP1 after short-term HU treatment. Interestingly, under these conditions, RAD51AP1-interacting proteins are enriched in components of the ubiquitin-proteasome system, including the subunits of the proteasome and proteasome receptors, ubiquitin ligases, and deubiquitylating enzymes (DUBs) [33]. In line with our findings [31] and with results from others [28, 32], Wagner *et al.* [33] showed that RAD51AP1 interacts with UAF1-USP1, but also with USP7 and USP11, DUBs with important roles in the cellular response to DNA damage and DNA replication stress [29, 43–45].

2.7. Posttranslational modifications of RAD51AP1

Further proteomic studies identified RAD51AP1 S120 as a target of ATR or ATM (ataxia telangiectasia mutated serine/threonine-protein kinase) following exposure of 293T cells to IR [46]. S120 is also phosphorylated after exposure of HeLa, U2OS or GM00130 B-lymphocyte cells to X- or γ -rays [47–49], and after etoposide treatment in U2OS cells [48]. Furthermore, S19, S20, S294 and S299 (in isoform 1) become phosphorylated after IR exposure [47, 48], although the responsible kinases remain to be identified. Interestingly, S294 and S299 lie within the C-terminal DNA binding domain of RAD51AP1 (Fig. 2), and it is tempting to speculate that phosphorylation of these 2 residues regulates the affinity of RAD51AP1 for DNA. RAD51AP1 is also acetylated and ubiquitylated (Fig. 2). Specifically, K132 and K269 (in isoform 1) are constitutively acetylated in HeLa cells [47], while K65, K123 and K343 become acetylated following either UV or X-ray exposure [47]. In U2OS cells, K343 is acetylated after treatment with etoposide [48]. K343 lies within the RAD51 interaction domain in RAD51AP1 [18], so it seems possible that K343 acetylation regulates the interaction with RAD51. K93, K123, and K156 are ubiquitylated upon treatment with either UV or IR, whereas K138 and K169 become ubiquitylated in response to IR only [47]. However, the phenotypic consequences of these ubiquitylations remain to be explored.

3. RAD51AP1 and cancer

3.1 Elevated RAD51AP1 may influence cancer development and progression

Cancer cells and precancerous lesions frequently contain aberrantly regulated DNA repair pathways [39, 50], and high expression of DNA repair genes has been described to be associated with metastasis [51–54]. With respect to studies on *RAD51AP1*, its transcript was found to be upregulated (5- to 26-fold) in primary hepatocellular carcinoma compared to normal liver tissue, pointing the investigators to suggest that elevated RAD51AP1 may play a role in hepatocarcinogenesis [55]. *RAD51AP1* transcript was also found to be elevated (4- to 16-fold) in 19/21 intrahepatic cholangiocarcinomas (ICCs) and suppression of *RAD51AP1* by shRNA resulted in growth suppression of cholangiocarcinoma cells, suggestive of the potential involvement of RAD51AP1 in promoting ICC development and/or progression [19]. *RAD51AP1* transcript was shown to be overexpressed in aggressive mantle cell lymphoma compared to indolent small lymphocytic lymphoma [17], in HPV-positive squamous cell carcinoma of the head and neck (3.6-fold) compared to normal oral epithelium [56], in ovarian cancer as a consequence of downregulated microRNA hsa-mir-140-3p [57, 58], and in basal and triple-negative breast cancers compared to normal mammary tissue [53, 59]. Consistent with a potential role for RAD51AP1 in metastatic disease [51, 52], high *RAD51AP1* expression was found to correlate with reduced time of survival of breast and ovarian cancer patients [58, 59]. Interestingly, sporadic cases of childhood papillary thyroid cancer (PTC) showed increased *RAD51AP1* message, while post-Chernobyl cases of PTC exhibited decreased *RAD51AP1*, indicating that the molecular events in PTC associated with low-dose IR exposure differ from that of patient-matched sporadic PTC [60]. Lastly, a retrospective analysis of microarray expression data revealed elevated expression of *RAD51AP1* in *BRCA1*-deficient compared to sporadic breast tumors [61], pointing towards genetic suppression of this breast cancer subtype by high RAD51AP1. Notably, Martin *et al.* also addressed if high levels of *RAD51AP1* in *BRCA1* tumor samples simply could reflect the higher proliferative index and/or fraction of cells in S-phase and conducted a gene-set enrichment analysis of the tumor data set employing 144 S-phase specific genes, but found no significant enrichment of these S-phase specific genes among *BRCA1* tumors [61].

Additional interesting observations of RAD51AP1's possible linkage to metastatic disease have been made. Elevated (> 4-fold) *RAD51AP1* transcript was detected in metastatic melanoma compared to metastasis-free melanoma [51, 52]. *RAD51AP1* expression was positively linked to expression of the stem cell marker CD271 and to mechanisms of therapy resistance in metastatic melanoma cells [52, 62]. Elevated expression of RAD51AP1 was also observed in a small set of brain metastasis [52]. Since RAD51AP1 is involved in mitigating replication stress [15, 16, 20, 33], it is tempting to speculate that its enhanced expression in metastatic tumors may stem from the requirement of metastatic cells to limit replication stress. We speculate that in some tumor cells and tissues, increased RAD51AP1 may be associated with a selective growth advantage, enabling early neoplastic cells and metastatic cells to overcome replication stress [38, 50] (Fig. 3). Nonetheless, the role of RAD51AP1 in tumor initiation and progression likely is cell-type specific, as reduced

RAD51AP1 stemming from CpG methylation of the *RAD51AP1* promoter region appears to be associated with prostate cancer [63].

3.2. RAD51AP1 as a potential therapeutic target

It has become increasingly apparent that lesions generated at DNA replication forks, as introduced by many anticancer drugs, are substrates for HR-mediated DNA repair [64]. Hence, HR-deficient tumors are sensitive to many DNA damaging agents that target replication forks. However, there are also many examples of the re- or over-expression of HR genes in tumor tissues, leading to anticancer drug resistance [64–66]. As described above, *RAD51AP1* could be one such example, and its expression may be linked to tumor resistance in response to anticancer treatment. Knockdown of *RAD51AP1* stunts the growth of intrahepatic cholangiocarcinoma cells [19], thus revealing a promising therapeutic avenue in *RAD51AP1* inhibition. Similarly, treatment of breast cancer stem cells with 5-azacytidine plus butyrate reduces *RAD51AP1* and cell proliferation [59]. Future work on *RAD51AP1* should focus on determining whether its downregulation or inhibition would improve the efficacy of cancer therapy. In this regard, targeting the RAD51 interaction domain on *RAD51AP1* may provide a new avenue of therapeutic intervention.

4. Concluding remarks

Since the discovery of *RAD51AP1* 20 years ago [7, 14], we have made significant progress towards a better understanding of its biochemical and cell biological attributes. However, we still lack knowledge of the precise molecular mechanisms by which *RAD51AP1* carries out the HR reaction in cells and stabilizes DNA replication forks, and how posttranslational modifications affect the activities and biology of *RAD51AP1*. In addition, the linkage between *RAD51AP1* and cancer initiation and progression is still poorly understood. Refined *in vivo* model systems using both *Rad51ap1* knockout and transgenic mice are required to better understand the organismal consequences of *Rad51ap1* deletion and overexpression in specific tissues. It will be particularly worthwhile to more extensively compare *RAD51AP1* expression in matched patient tumor and control samples to establish its potential as a tumor biomarker. Filling the knowledge gap in the aforementioned areas will lead to a better understanding of the complexities associated with the resolution of DNA recombination intermediates and will also enhance the prospects for targeted therapies of cancers with high *RAD51AP1*.

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Highlights

- RAD51AP1 is a DNA-binding protein that stimulates RAD51 activity.
- RAD51AP1 protects against DNA damage and is required for replication fork and genome stability.
- *RAD51AP1* expression may be linked to cancer development and progression.
- RAD51AP1 has the potential to become a tumor biomarker.
- RAD51AP1 may provide a new target in cancer therapy.

Fig. 1. RAD51AP1 in HR DNA repair and functional domains. **(A)** The involvement of RAD51AP1 in the HR reaction is confined to synaptic complex formation and strand-invasion, HR stages downstream of presynaptic filament formation. **(B)** Cartoon of the RAD51AP1 protein with mapped domains of DNA and protein interactions, as indicated. For details see text.

Isoform 1	MVRPVRHKKPVNYSQFDHSDSDDDFVSATVPLNKKSR	TAPKELKQDKPKPNLNNLRKEEI	60
Isoform 2	MVRPVRHKKPVNYSQFDHSDSDDDFVSATVPLNKKSR	TAPKELKQDKPKPNLNNLRKEEI	60
DNA			
Isoform 1	PVQERTPKKRLPEGTF	SIPASAVPCTKMALDDKLYQRDLEVALALSVKELPTVTTNVQNS	120
Isoform 2	PVQERTPKKR-----	MALDDKLYQRDLEVALALSVKELPTVTTNVQNS	103
Isoform 1	*QDKSIEKHGSSKIETM	NKSPHISNCSVASDYLDLDKITVEDDVGGVQGRKAASKAAQ	180
Isoform 2	QDKSIEKHGSSKIETM	NKSPHISNCSVASDYLDLDKITVEDDVGGVQGRKAASKAAQ	163
Isoform 1	RKILLEGSDGDSANDTE	PDFAPGEDSEDDSDFCESDNDEDFSMRKS	240
Isoform 2	RKILLEGSDGDSANDTE	PDFAPGEDSEDDSDFCESDNDEDFSMRKS	223
Isoform 1	SPVEKKEKKSKSKCNALVTSVDS	SAPAAVSESQSLPKKVSLSDDTTRKPLEIRSPSAESK	300
Isoform 2	SPVEKKEKKSKSKCNALVTSVDS	SAPAAVSESQSLPKKVSLSDDTTRKPLEIRSPSAESK	283
DNA (I)		DNA (II)	
Isoform 1	KPKWVPPAASGGSRSSSSPLVVSVKSPNQSLRLGLSRLARVPLHPNATST	352	
Isoform 2	KPKWVPPAASGGSRSSSSPLVVSVKSPNQSLRLGLSRLARVPLHPNATST	335	
RAD51			

Fig. 2. ClustalW sequence alignment of the two prevalent isoforms of RAD51AP1 (<http://www.proteinatlas.org/>) with identified residues for posttranslational modification [33, 46–49] highlighted, as follows: phosphorylation (green), acetylation (red), and ubiquitylation (blue). Asterisk on K123 (isoform 1) indicates that this residue is either acetylated or ubiquitylated in response to DNA damage [47]. The N-terminally located and the 2 identified parts (DNA (I) and DNA (II)) of the C-terminally located DNA-binding domains [22] are underlined by a solid line. The RAD51 interaction domain is also underlined.

Fig. 3. Model to explain why elevated *RAD51AP1*/RAD51AP1 may provide a selective advantage during tumor initiation and metastasis, when endogenous levels of DNA damage are high [38, 39, 50, 51, 54]. BM: basement membrane; BV: blood vessel.