The in vivo role of Rev1 in mutagenesis and carcinogenesis

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Abstract

Translesion synthesis (TLS) is an error-prone pathway required to overcome replication blockage by DNA damage. Aberrant activation of TLS has been suggested to play a role in tumorigenesis by promoting genetic mutations. However, the precise molecular mechanisms underlying TLS-mediated tumorigenesis in vivo remain unclear. Rev1 is a member of the Y family polymerases and plays a key role in the TLS pathway. Here we introduce the existing to date Rev1-mutated mouse models, including the Rev1 transgenic (Tg) mouse model generated in our laboratory. We give an overview of the current knowledge on how different disruptions in Rev1 functions impact mutagenesis and the suggested molecular mechanisms underlying these effects. We summarize the available data from ours and others’ in vivo studies on the role of Rev1 in the initiation and promotion of cancer, emphasizing how Rev1-mutated mouse models can be used as complementary tools for future research.

Keywords: Translesion synthesis, Rev1, Mutagenesis, Tumorigenesis

Introduction

The genome of all living organisms is continually attacked by endogenous and exogenous genotoxic DNA damaging agents, such as metabolic processes, UV light, ionizing radiation, etc., and various DNA repair pathways have evolved to protect genome integrity. TLS is a mechanism of DNA damage tolerance involving specialized DNA polymerases with the capacity to replicate across damaged DNA template [1–5]. Mammalian TLS polymerases include Y-family polymerases (Pol η, Pol ι, Pol κ, and Rev1), 1 B-family polymerase (Pol ζ: Rev3L/ Rev7/ PolD2/ PolD3), 2 A-family polymerases (Pol θ and Pol ν), and three X-family polymerases (Pol μ, Pol λ, and Pol β) [6].

Rev1, in tandem with Pol ζ, stands out as a central player in error-prone TLS [3, 4, 7]. Rev1 is a deoxyacytidyl transferase which incorporates deoxycytosines opposite structurally diverse damaged nucleotides, such as 6O-meG, a guanine with a large adduct at the C8 or N2 position, or abasic sites [8–17]. However, the most important function of Rev1 in error-prone TLS is regulatory rather than catalytic by recruiting the Y-family polymerases Pol η, Pol ι and Pol κ and the B-family Pol ζ, which interacts with Rev1 via its Rev7 component [18–23]. Inhibition of Rev1 results in enhanced sensitivity and reduced mutation frequencies in response to DNA-damaging agents, such as UV light, hydrogen peroxide, cisplatin, and X-rays [24–28].

Mutations in human REV1 have been detected in a minority of tumors [29], and single-nucleotide polymorphisms (SNPs) in the hREV1 gene have been linked to various types of human cancer [30–32]. As with other TLS polymerases, upregulation of hREV1 is associated with the pathogenesis of human cancer [33]. Since hREV1 plays a critical role in TLS, and TLS contributes to the pathogenesis of tumors and to drug resistance by promoting tolerance of DNA damage, targeting hREV1 might be a promising approach for improving the outcome of chemotherapy. Recently, Wojtaszek J et al. reported that a small-molecule inhibitor of mutagenic translesion DNA synthesis (JH-RE-06), which disrupts the interaction between hREV1 and/or REV7, sensitizes cancer cells to cisplatin in vitro and in vivo [34].

Currently, despite the fact that several Rev1-mutated mouse models have been established, there is very little data regarding the role of Rev1 dysregulations in carcinogenesis in vivo (Table 1). Here we introduce four complementary Rev1 mutated mouse models, including the Rev1 Tg mouse model generated in our laboratory,
and we discuss their distinctive advantages for carcinogenesis research. This is the review based on the authors’ presentation at the Open Symposium of the Japanese Environmental Mutagen Society (JEMS) in 2017 [35].

**Rev1 KO mice**

*Rev1* knockout (KO) mice were first generated with the objective of investigating the role of Rev1 in somatic hypermutation (SHM) [36]. The authors reported that *Rev1* KO mice showed delayed growth, a shortened lifespan and were nearly infertile [36]. It should be noted that in addition to SHM, Rev1 is also involved in class switch DNA recombination (CSR), and both SHM and CSR are critical for maturation of the antibody response [37–39]. SHM and CSR are initiated by activation-induced cytidine deaminase (AID), which deaminates deoxycytosine (dC) residues to yield deoxyuridine (dU): deoxyguanine (dG) mispairs. These mispairs then trigger DNA repair processes facilitated by Uracil DNA glycosylase (Ung), Mismatch repair (MMR) proteins, and Rev1 [40]. SHM analysis demonstrated that mutation frequency and distribution were similar in B-cells from *Rev1* KO and wild type mice [36]. In contrast, the mutation spectra were significantly altered by the deletion of *Rev1*. An almost complete absence of C to G transversions was observed in *Rev1* KO cells, accompanied by a moderate decrease in G to C transversions and an increase in A to T substitutions similar to Ung deficiency. Since the induction of A to T mutations is highly dependent on Pol η, it is possible that the Rev1 defect results in increased access of Pol η to sites of DNA lesions. It has been reported that G: C to C: G transversions during SHM are generated downstream of two pathways: Ung2-dependent/Msh2-independent and Ung2/Msh2-dependent pathways [41]. Rev1 is indispensable in the former pathway and it plays the main role in the latter pathway, although in this case it can be replaced by other TLS polymerases. During CSR Rev1 recruits Ung to switch (S) regions and enhances dU glycosylation [42]. Nevertheless, Rev1 deficiency only slightly reduces CSR. In contrast, double *Rev1*/Msh2 defects lead to ablation of CSR, similarly to double *Ung*/Msh2 deficiency. CSR analysis has revealed that Rev1 exerts its functions in CSR via its non-catalytic properties.

Mouse embryonic fibroblasts (MEF) derived from *Rev1* KO mice have been reported to proliferate more poorly than wild-type cells [43]. Similarly, *Rev1* KO hematopoietic stem cells display competitive and proliferative disadvantage [44]. Furthermore, the additional disruption of *Xpc*, which is essential for global-genome nucleotide excision repair (ggNER), results in progressive loss of bone marrow, and fatal aplastic anemia between 3 and 4 months of age [44]. This finding suggests that Rev1-dependent TLS protects the genomic and functional integrity of the hematopoietic system in coordination with ggNER. In summary, the currently existing data is limited to genome instability and table

| Mouse model | Defect            | Growth | Fertility | Life span | Tumor                                    | References       |
|-------------|-------------------|--------|-----------|-----------|-----------------------------------------|------------------|
| *Rev1* KO   | *Rev1* deficient   | Delayed| Near-infertile | Reduced   | unknown                                 | [36, 44]         |
| *Rev1*ΔN   | *Rev1* catalytic activity | Normal | Normal    | Normal    | not accelerated intestinal adenoma (MNU-induced)* | [45]             |
| *Rev1*ΔBRCT | *BRCT* region      | Normal | Normal    | Normal    | accelerated skin cancer (UV induced)    | [46]             |
| *Rev1* Tg  | *Rev1* overexpression | Normal | Normal    | Normal    | accelerated hepatocellular carcinoma (AOM induced) accelerated thymic lymphoma and intestinal adenoma (MNU-induced)* | [47,48]          |

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**Fig. 1** Structure of Rev1

BRCT: BRCA1 C terminus (BRCT) domain  
Y-pol CD: Y-family DNA polymerase catalytic domain 
UBM: ubiquitin binding motif  
YP CR: Y-family DNA polymerase contacting region
mutagenesis, but carcinogenesis studies on Rev1 KO mice have not been published, and the impact of Rev1 deficiency on cancer development in vivo remains unknown.

Rev1AA mice

Rev1AA mice are defective specifically for Rev1 catalytic activity due to mutations in a Y-family DNA polymerase catalytic domain of Rev1 (Fig. 1) [45]. Rev1AA mice develop normally and are fertile. SHM analysis has demonstrated that B-cells from Rev1AA mice are characterized by reduced overall mutation frequency and decreased mutagenesis at both G:C and A:T base pairs. This contrasts the abovementioned increase in A to T substitutions in Rev1 KO mice, and one likely explanation is that Rev1AA might inhibit the access of Pol η to sites of DNA lesions by remaining at the abasic site. Carcinogenesis study conducted in our laboratory suggests that Rev1AA does not affect chemically-induced mutagenesis and carcinogenesis (Sasatani et al., manuscript in preparation). Thus, the catalytic domain of Rev1 appears to be dispensable for either normal development or tumorigenesis.

Rev1 BRCA1 C terminus (BRCT) mice

Rev1BRCT mice carry a deletion in the BRCT domain of Rev1, which, however, retains catalytic function (Fig. 1) [46]. Rev1BRCT exhibit a normal phenotype spontaneously, while being sensitive to exogenous DNA-damaging factors and exhibiting lower levels of ultraviolet C (UVC)-induced mutagenesis. Interestingly, despite the reduced mutagenesis, Rev1BRCT;Xpc KO mice have been shown to be more vulnerable to skin carcinogenesis than Xpc KO mice. The authors concluded that this paradoxical phenotype was due to the induction of inflammatory hyperplasia that facilitates tumor promotion. Thus, the Rev1BRCT mouse model is useful for interrogating the distinctive roles of Rev1 in the initiation versus the promotion step of tumor development.

Rev1 Tg mice

Rev1 Tg mice were generated in our laboratory by using the metallothionein promoter 1 (MT-1) to achieve inducible expression [47]. We found that the Rev1 transgene was expressed at low levels in the liver and kidney, but at dramatically higher levels in the thymus, spleen, and lymph nodes. In order to determine whether overexpression of Rev1 would influence spontaneous tumor initiation and progression, we monitored cohorts of Wt and Rev1 Tg mice over their lifespan (> 2 years). No significant effect on overall survival or tumor incidence was observed, suggesting that overexpression of Rev1 by itself is not sufficient to stimulate tumorigenesis. However, our study revealed that overexpression of Rev1 promotes development of chemically-induced tumors, namely azoxymethane (AOM)-induced hepatocellular carcinoma, and N-methyl-N-nitrosourea (MNU)-induced thymic lymphoma and intestinal adenomas (Sasatani et al., manuscript in preparation) [47, 48]. Furthermore, in a comparative analysis of MNU-induced carcinogenesis in Rev1 Tg (Homo) mice, which are homozygous (Tg+/Tg+) for the Rev1 transgene, versus heterozygous Rev1 Tg mice, we provided evidence that Rev1 overexpression accelerates tumorigenesis in proportion to the Rev1 expression level. Following MNU treatment, we observed enhanced mutagenesis and suppressed apoptosis in proportion to the level of Rev1 overexpression.

Our data implies that overexpression of Rev1 promotes mutagenic TLS to safeguard replication on damaged templates, consequently inhibiting apoptosis and accelerating tumorigenesis (Fig. 2) (Table 2). Although the role of REV1 overexpression in human carcinogenesis remains poorly understood, human cells overexpressing REV1 exhibit a comparable to the abovementioned

![Fig. 2](image_url) Model of accelerated chemically-induced tumorigenesis mediated by Rev1 overexpression. Overexpressed Rev1 suppresses apoptosis and increases the mutation frequency after the treatment of chemical reagents. The surviving fraction of mutated cells was higher under Rev1 overexpression, resulting in acceleration of carcinogenesis.
phenotype, with enhanced mutation frequency and hindered cell death, therefore, it can be clearly stated that regulation of Rev1 levels is required for maintaining genomic stability and tumor suppression (Table 2) [47].

Table 2

| Tumorgensis | Rev1 Tg mice | REV1 overexpression Human cell line |
|-------------|--------------|-----------------------------------|
| Mutation frequency | ↑ | ↑ |
| Cell death | ↓ | ↓ |
| Sister chromatid exchange | N.D. | ↓ |

ND not detected

Conclusions

Rev1 is a member of the TLS polymerase family and plays a key role in this mutagenic pathway, which allows the bypass of modified DNA bases and respectively, facilitates proliferation even in the presence of extensive DNA damage, such as during chemotherapy. Therefore, inhibition of the TLS pathway may be a promising strategy to tackle the problem of resistance to chemotherapy and to improve the therapeutic outcome.

Here we have introduced several mouse models with disruptions in Rev1 functions, including the Rev1 Tg mouse model generated in our laboratory. Recently we have reported that in Rev1 Tg mice the elevated Rev1 expression allows cells with mutations to survive after DNA damages, resulting in an acceleration of tumorigenesis [47, 48]. However, in vivo data like this is almost completely absent from the literature. We hope that data from studies employing Rev1-mutated mouse models will soon become available and will help us elucidate the mechanisms of Rev1-mediated tumorigenesis and chemotherapy resistance, so that we can in the future harness the therapeutic potential of TLS targeting.

Abbreviations

Rev1: Reversionless 1; SHM: Somatic hypermutation; Tg: Transgenic; TLS: Translesion synthesis

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Authors’ contributions

MS, EZ and KK contributed to writing the manuscript. All authors approved the final manuscript.

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Not applicable.

Consent for publication

The authors declare that they have no competing interests.

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