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hRev7, putative subunit of hPolζ, plays a critical role in survival, induction of mutations, and progression through S-phase, of $UV_{(254\,nm)}$-irradiated human fibroblasts☆

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ABSTRACT

Translesion synthesis (TLS) refers to mechanisms by which specialized DNA polymerases incorporate nucleotides opposite fork-blocking lesions and extend replication until standard replicative polymerases take over. The first eukaryotic TLS polymerase discovered, *S. cerevisiae* Polζ, consists of catalytic subunit Rev3 and non-catalytic subunit Rev7. Human homologs of these two proteins have been identified. Studies by Lawrence, Maher, and colleagues comparing $UV_{(254\,nm)}$-irradiated human fibroblast cell strains expressing high levels of *hRev3* antisense to their normal parental strains demonstrated that there was no difference in cell survival, but that the frequency of UV-induced mutations in the derivative strains was 10-fold lower than that of the parental strains, indicating that hRev3 plays a critical role in such mutagenesis. To examine the role of hRev7 in TLS, we generated human fibroblasts expressing *hRev7* siRNA, identified two derivative cell strains with significantly reduced levels of hRev7, and compared them to their parental strain and a vector control for cell survival, induction of mutations, and ability to traverse the cell cycle following exposure to UV radiation. Cells with reduced hRev7 were ~2-times more sensitive to UV-induced cytotoxicity than the controls, indicating that unlike hRev3, hRev7 plays a protective role for cells exposed to UV radiation. When these cell strains were assayed for the frequency of mutations induced by UV in their *HPRT* gene, cell stains with reduced hRev7 were 5-times less sensitive to UV-induced mutagenesis than control strains. In addition, when these four strains were synchronized at the G1/S border, released from the block, UV-irradiated, and allowed to traverse the cell cycle, the rate of progression through S-phase of the cell strains with reduced hRev7 was significantly slower than that of the control strains. These data strongly support the hypothesis that hRev7 is required for TLS past UV-photoproducts, and together with hRev3, comprise hPolζ.

☆ *Disclosure*: Materials used were not from other published papers. The data in this manuscript have not been published elsewhere.

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1. Introduction

Human cells are continually exposed to endogenous and exogenous DNA damaging agents, many of which create fork-blocking lesions. If DNA replication past such lesions cannot take place, this can lead to cell death, nevertheless replication past such lesions can result in mutations. Because mutations play a crucial causal role in the development of cancer, it is important to examine processes that produce them.

Human cells have efficient, error-free repair pathways for excising DNA fork-blocking lesions from either strand of their DNA. They also possess cell cycle checkpoints [1], some of which, when activated, provide additional time for excision repair to occur before the replicative polymerases encounter fork-blocking lesions, such as UV-induced pyrimidine dimers. In spite of these protective processes, replication forks still encounter lesions. Cells have evolved damage tolerance mechanisms to cope with such lesions, viz., translesion synthesis and damage avoidance pathways. Such methods of dealing with fork-blocking damage have been, and continue to be actively examined. Overviews summarizing in detail such areas of research can be found in reference [2].

Translesion synthesis in both prokaryotes and eukaryotes involves specialized DNA polymerases capable of incorporating nucleotides directly across from fork-blocking DNA lesions. This insertion step can be error-free or error-prone, depending upon (1) the type of DNA lesion encountered, (2) the specialized polymerases involved, and (3) the sequence context surrounding the site of the damage. Insertion of a nucleotide or nucleotides by one or other such polymerases is followed by extension, i.e., the addition of nucleotides beyond the site of the blocking lesion. This latter step also involves TLS DNA polymerases. Such extension beyond the damage is necessary if the high fidelity replicative DNA polymerases are to resume their function. Thus, TLS is a two-step process whereby specialized DNA polymerases, with relaxed fidelity, incorporate and/or extend nucleotides at sites of fork-blocking DNA damage, allowing DNA replication to continue, but often introducing mutations.

Reports and summaries of the discovery of many translesion synthesis polymerases, first in *S. cerevisiae*, and later in mammalian cells, can be found in the cited references (see for example, [3–6]). However, many aspects still remain to be clarified. Polζ was found using *S. cerevisiae* cells whose specific mutated phenotypes could not be reverted to wild type by exposure to mutagenic agents. Genes that complemented the deficiencies in such strains of *S. cerevisiae*, i.e., allowed them to revert, were identified and subsequently shown to code for proteins that allow replication past fork-blocking DNA damage [7,8]. For example, the yeast Rev3 protein was found to exhibit polymerase activity in primer extension assays *in vitro*. The addition of yeast Rev7 to such assays enhanced the polymerase activity of Rev3 over 20-fold. Together, Rev3 and Rev7 were recognized as constituting yeast Polζ [9].

Genes coding for the human homologs of yeast Rev3 [10–13] and Rev7 [14] were subsequently identified. By using antisense directed against *hRev3* mRNA, Lawrence, Maher, and their colleagues [10,15] demonstrated that hRev3, the putative catalytic subunit of hPolζ, is critically involved in generating UV-induced mutations in diploid human fibroblasts. These results indicate that hRev3 is essential for a mutagenic process involving DNA lesions that interfere with replication, just as yeast Rev3 is. The hRev3 protein of human cells, a predicted 353 kDa molecule [10], has not yet been isolated, but the non-catalytic subunit, hRev7, a much smaller molecule, has been isolated [14].

The present study was carried out to test the hypothesis that hRev7, the putative non-catalytic subunit of hPolζ, is also involved in human cell mutagenesis. For such a study, an approach similar to that used for investigating the role of hRev3 was employed, but instead of using antisense RNA to block expression of the target protein, siRNA against hRev7 was used to reduce the level of this protein in human fibroblasts. The fact that antibodies capable of detecting very low levels of hRev7 protein were available allowed us to identify independent cell strains in which the level of hRev7 protein had been greatly reduced by siRNA. Comparing the results obtained using these cell strains with those obtained using their parental human fibroblasts and a vector control strain allowed us to demonstrate that hRev7, the non-catalytic subunit of hPolζ, plays a role in the survival of UV-irradiated human cells, and has a significant role in UV-induced mutagenesis. Using these human cell strains, we also demonstrated that reduction in the expression of hRev7 impedes the cells' ability to progress through S-phase.

2. Materials and methods

2.1. Cell culture

Cells were grown in Eagle's minimum essential medium, supplemented with 0.2 mM L-aspartic acid, 0.2 mM L-serine, 1 mM sodium pyruvate, 10% supplemented calf serum (HyClone), 100 units/ml penicillin, 100 μg/ml streptomycin, 1 μg/ml hydrocortisone and 1 μg/ml tetracycline.

2.2. Cell strains

The parental human cell strain used for these studies, designated MSU-1.2.9N.58, was derived from the cell strain MSU-1.2, a spontaneous derivative of the infinite life span cell strain MSU-1.1, whose origin from the foreskin-derived from a normal neonate and subsequent acquisition of an unlimited life span in culture has been described [16]. MSU-1.2 cells are near-diploid, chromosomally-stable, and grow vigorously as a result of expressing their endogenous gene for platelet-derived growth factor.

2.3. Derivation of cell strains with reduced hRev7

Oligonucleotides designed to target *hRev7* mRNA were annealed to a complementary oligonucleotide according to the manufacturer's protocol (Ambion). Using T4 DNA ligase (New England Biolabs), annealed-oligonucleotides were ligated into the pSilencer3.1 vector (Ambion), which includes the gene coding for puromycin resistance, and purified. The parental MSU-1.2.9N.58 cells were transfected with such siRNA vectors, using Lipofectamine (Invitrogen) according to the manufac-