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Brief report

Validation of XP-C pathogenic variations in archival material from a live XP patient

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ARTICLE INFO

Article history:
Received 21 September 2006
Accepted 25 September 2006
Published on line 3 November 2006

Keywords:
Nucleotide excision repair
Archival specimen
DNA sequenceing
Mutation detection

Abbreviations:
XP, xeroderma pigmentosum; NER, nucleotide excision repair; NMD, nonsense-mediated decay; qRT-PCR, quantitative reverse transcription polymerase chain reaction

ABSTRACT

Xeroderma pigmentosum (XP) genetic complementation group C (XP-C) is the most common form of the disease worldwide. Thirty-four distinct genetic defects have been identified in 45 XP-C patients. Further identification of such defects and the frequency of their occurrence offers the potential of generating diagnostic and prognostic molecular screening panels. Archival material (such as formalin-fixed paraffin embedded skin) may be useful for the identification of novel genetic variations and for documenting the frequency of individual genetic defects in patients who are no longer available for study. However, the use of archival material precludes direct analysis of changes in the mRNA resulting from genomic changes. The serendipitous reacquisition of an XP individual in whom genetic defects were previously characterized in archival material allowed confirmation of the defects as well as a direct analysis of the consequences of these defects on mRNA, mRNA expression and on cellular phenotypes.

1. Introduction

Xeroderma pigmentosum (XP) is a rare hereditary disorder characterized by sun sensitivity and a predisposition to cancer. XP individuals manifest considerable phenotypic variability, presumably reflecting the effects of different mutant alleles detected in any one of eight unlinked genes for the disease [1]. Seven of these genes encode proteins that are involved in the process of nucleotide excision repair (NER) and one encodes a specialized DNA polymerase [1]. Seven of these genes encode proteins that are involved in the process of nucleotide excision repair (NER) and one encodes a specialized DNA polymerase (Polη) implicated in translesion DNA synthesis across sites of UV radiation-induced base damage [2–4]. Individual mutations in any of the genes implicated in XP can result in a range of cellular phenotypes. Indeed, such genomic alterations in the *XPB* or *XPD* genes can result in any of three phenotypically distinct disorders (i.e. xeroderma pigmentosum, Cockayne syndrome or trichothiodystrophy).

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doi:10.1016/j.dnarep.2006.09.009

Historically, somatic cell genetics has been employed to determine which of the multiple XP-related genes is mutated in any single case. In such studies cells from individuals diagnosed clinically are fused to those from patients in known genetic complementation groups and the resulting hybrid cells examined for correction of cellular phenotypes such as UV radiation sensitivity [5]. More recently co-transfection of expression plasmids containing the coding sequence of each of the known XP-associated genes together with a reporter plasmid carrying UV radiation-induced base damage has largely replaced this assay [6]. Both of these techniques require live cells derived from skin biopsies. However, assignment of a particular genetic complementation group in archival material requires the isolation of DNA from such material and locating pathogenic variations in one of the multiple XP genes. Such studies necessarily preclude in depth analysis of individual pathogenic variations. In particular, it in not possible to unequivocally assign individual pathogenic variations to different alleles, nor is it possible to examine their effects at the mRNA and/or protein levels. In a separate report we described the molecular characterization of archival material from an individual diagnosed clinically with XP. This analysis revealed the presence of two pathogenic variations in the XPC gene that are expected to result in premature termination of transcription, thus dramatically reducing steady-state levels of *XPC* mRNA due to nonsense-mediated decay (NMD). After these studies were completed, we were able to fortuitously gain access to the living patient from whom the original archival material was originally obtained. Here we describe the further characterization of patient XP1UTSW using a viable cell line obtained by skin biopsy. Amplification of the complete cDNA demonstrates that the two variant alleles detected in the patient are indeed separate alleles. Additionally, qRT-PCR results with the viable cells confirmed reduced levels of mRNA predicted from the analysis of formalin-fixed paraffin-embedded tissue, supporting the notion that archival samples constitute valuable research reagents for such studies. Additionally, survival curves performed with the viable cells confirm that the patient is sensitive to killing by UV-irradiation in the range typical of XP-C patients.

2. Materials and methods

2.1. Cell culture and sequencing

Skin (S05-5125) and skin tumor (T05-5124) tissue was placed in culture without collagenase and grown in DMEM supplemented with antibiotic/antimycotic (Invitrogen, Carlsbad, CA) and 15% FBS at 37 °C in the presence of 5% CO_2. VA is an SV-40 transformed cell line derived from WI-38 cells and XP4PA(sv) is an SV-40 transformed XPC cell line. The control fibroblast used for the survival curve and qRT-PCR was GM01604. RNA was isolated using a Purescript and genomic DNA by Puregene (Gentra, Minneapolis, MN) according to the manufacturer's instructions. RNA was reverse transcribed and amplified by PCR using the SuperScript One-Step RT-PCR system (Invitrogen, Carlsbad, CA). Amplification primers were from exon 1 and exon 15 (XPCc-F1, CGAAGTGGAATTTGCCCAGAC and XPCm-B1, CCAGGCAGTCAGGAGCACGTC) ligated into the pGEM-T Easy Vector (Promega, San Luis Obispo, CA) and transformed into bacteria. Plasmid was isolated and sequenced directly using vector specific primers M13-F and M13-R and XPC primers: XPCc-F3, GCTGGTATTGTCTCTACAGCCAATTC, XPCc-F4, GAGGTGTTCTGTGAGCAGGAGG, XPCc-B1, GCCCTCCGAAGATATGTCTCAAAC, XPCc-B3, TGTCAATGCCCACCACATAGG, and XPCm-F1, GGAGTTTGAGACATATCTTCGGAGG. Products were rum on an ABI3100 Automated DNA Sequencer. Sequence was analyzed using the reference sequence from UCSC GenPath Database Genome March 2006 assembly (http://genome.cse.ucsc.edu/). This reference sequence has remained unchanged since the July 2003 assembly.

Cells for the survival curves were exposed to UV-irradiation in the appropriate dose and allowed to form colonies for 10 days to 2 weeks. Colonies were washed with PBS, fixed with 95% ethanol and stained with crystal violet solution (0.25%).

2.2. Quantitative RT-PCR (qRT-PCR)

RNA (10 ng) was reverse transcribed and amplified using Brilliant® QRT-PCR one-step kit in a MX3000P Q-PCR thermocycler (Stratagene, La Jolla, CA). The primers for XPC spanned exons 3–4 (XPCe3-F, AGAAGGCACACCATCTGAAGAGAG and XPCe4-R, CCCAGCACAGGCTCACTAAGTTC). Primers from a control gene, DOCK5 (DOCK5-F, TGCTCAGTGGCATCGTGGAC and DOCK5-R, GCTCAACCTTCTCCTGGTCTTCAG) resulted in similar product size and amplification efficiency after validation (Stratagene La Jolla, CA, User bulletin #2). qRT-PCRs were run in triplicate on at least three occasions with different RNA preparations. Results were analyzed according to the manufacturers instructions using the comparative Ct method.

3. Results

3.1. UV-survival

Fibroblasts from the patient XP1UTSW [7], a control cell line and an XPC cell line were grown in culture and plated in appropriate number for a UV-survival study and irradiated with doses of UV light late the same day. When colonies were of sufficient size, plates were fixed and colonies counted. Survival was graphed on a log scale as percent of colonies that survived based on the control not exposed to UV-irradiation (Fig. 1). The S05-5125 cell line from patient XP1UTSW appears to be slightly more sensitive to UV-irradiation than the known XP-C cell line

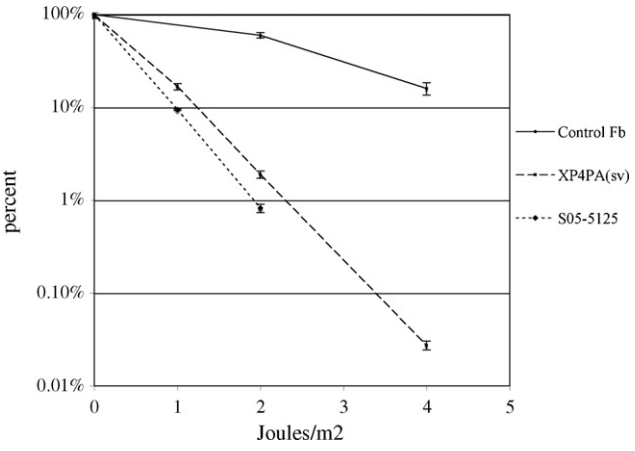

Fig. 1 – UV-survival curve. Cells were plated at densities resulting in 100–200 colonies on each plate, performed in triplicate and repeated three times. Results are plotted as percent survival based on the unirradiated control on a log scale against the dose of UV light as J/m^2. A typical example is shown with error bars indicating the standard deviations.