Biochemistry and Biophysics Reports 40 (2024) 101875

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Co-infection of HSV-1 amplicons containing the *XPC* gene and a human artificial chromosome vector into primary XPC deficient fibroblast cells

Daniela Moralli [a], Avi Smith [b], Jonathan Garlick [b], Kenneth H. Kraemer [c], Zoia L. Monaco [d,*]

[a] *Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, UK*
[b] *Department of Diagnostic Sciences, Tufts University School of Dental Medicine, 55 Kneeland Street, Boston, MA, 02111, USA*
[c] *Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 37 Rom 4002, Bethesda, MD, 20892, USA*
[d] *Tufts University, Department of Biomedical Engineering, 4 Colby Street, Medford, 02155, USA*

ARTICLE INFO

Keywords:
Xeroderma pigmentosum (XP)
XPC gene
Human artificial chromosome (HAC)
Herpes simplex Virus-1 (HSV-1)
Skin cancer and DNA repair

ABSTRACT

Gene therapy for xeroderma pigmentosum (XP), a rare, recessive DNA repair disease, has been considered since defects in XP genes result in severe and debilitating symptoms. Mutations in the *XPC* DNA repair gene result in a more that 1000-fold increased sensitivity to sunlight-induced skin cancer. The *XPC* gene is large (33 Kb) and the entire genomic locus is a difficult candidate for many gene therapy vectors to incorporate into their system by conventional cloning. Artificial chromosome vectors were developed to accommodate large genes and their regulatory sequences to allow full gene expression in cells. The HSV-1 human artificial chromosome (HAC) vectors we previously generated incorporated genes up to 100 Kb in a single vector. Subsequently, we modified the system to allow larger (>100 Kb) DNA gene sequences to be introduced by simultaneously infecting cells with two separate HSV-1 vector particles, one containing DNA required for HAC formation and the other with the desired gene. Following transduction, recombination of DNA formed a gene expressing HAC *in vitro*. The dual transduction system was successful for introduction and expression of the HPRT gene in human 3D engineered tissues and stem cells. In this study, we report the *XPC* gene delivery and transient gene expression via the dual transduction system in human cultured fibrosarcoma (HT1080) and primary XPC deficient patient cells.

1. Introduction

Xeroderma pigmentosum (XP) is a rare, (about 1 per million in the US and Europe) autosomal recessive disorder caused by defects in the nucleotide excision repair (NER) genes (*XP-A to XP-G*) and in the translesion DNA synthesis gene *PolH* (XP variant) [1]. Many XP patients experience acute burning on minimal sun exposure, multiple pigmented lesions and a greater than 1000-fold increased risk of sunlight induced skin cancer including basal cell carcinomas, squamous cell carcinomas, and melanomas. Sunlight induced damage to the eye (lids, cornea and conjunctiva) can occur, leading to vision loss and ocular neoplasms and patients are required to shield their skin and eyes to prevent UV exposure. Some patients have progressive neurological defects with associated developmental problems in young children [1]. In the United States, mutations in the NER *XPC* gene are the most common, with patients exhibiting symptoms including cancer of the skin and eyes. The *XPC* gene is located on chromosome 3p25, and the gene locus is approximately 33 kb [2]. This gene is essential for the rapid recruitment of NER proteins at sites of DNA photodamage [3], where the XPC protein forms a complex with the RAD 23 gene and the centrosomal protein Centrin to initiate NER [4].

Progress in developing therapeutic strategies have been slow. Small cancerous areas of the skin can be excised and replaced with skin autografts, though this is not a feasible therapy for much larger areas or a whole-body approach. Strategies for studying gene expression with the aim of therapy have included using retrovirus vectors to deliver the *XPC* cDNA to keratinocytes [5]. For clinical purposes, use of retrovirus is problematic. The control of gene expression under non-physiological promoters in the retrovirus vector may result in a different expression pattern than the *XPC* physiological promoter, and there is a theoretical risk of mutagenesis and activation of oncogenes due to integration of the retrovirus into the genome. Applying antibiotics for selecting the retrovirus vector in cells can lead to cell senescence and apoptosis [6]. A targeted approach *in situ* via gene editing using meganucleases or talen

* Corresponding author.
E-mail address: zoia.monaco@tufts.edu (Z.L. Monaco).

https://doi.org/10.1016/j.bbrep.2024.101875
Received 16 September 2024; Received in revised form 27 October 2024; Accepted 12 November 2024
2405-5808/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

nucleases was successfully reported [7]. Meganucleases require demethylation of the genome, which is not clinically practical for therapeutic purposes, though editing with Talen nucleases was successful under methylated conditions and a more viable approach. Another strategy utilized oligonucleotides to correct the mutation in the XP genes, though this study requires further validation [8].

Incorporation of the entire *XPC* gene locus into a human artificial chromosome (HAC) vector may provide conditions for appropriate regulation of the *XPC* gene under the control of its own physiological promoter. The research in this HAC study is based on earlier work where we described the development of an efficient gene expressing HAC system using Herpes Simplex Virus-1 (HSV-1) amplicons for delivery of large genes in HAC vectors, for complementation of gene deficiencies and correction of genetic defects in human cells [9,10]. This technique was successful in human stem and induced pluripotent stem (iPS) cells. It was based on cloning large genes or gene loci (>10 kb) into a single HAC vector (known as the bottom-up approach) for transfer to cells using amplicons [11]. Since that time, we developed a simpler system without large scale cloning. This included simultaneous co-infection of different gene containing vector HSV-1 amplicons into human cultured (fibrosarcoma, HT1080), embryonic stem and iPS cells and the successful selection of gene containing HACs [12]. One HSV-1 amplicon contained DNA required for HAC formation and the other contained the desired gene. This was an easier approach involving fewer modification steps and allows for introduction of several genes simultaneously into cells for studying gene expression from a HAC.

In this study, we utilized the co-infection strategy with HSV-1 amplicons to introduce the entire *XPC* gene locus into patient XPC deficient primary fibroblasts. The aim was to develop a useful model system for not only monitoring *XPC* gene expression from a HAC vector, but also investigating the conditions required for transducing primary fibroblasts with HSV-1 HAC amplicons.

2. Materials and methods

2.1. HAC vectors

The *XPC* HSV-1 amplicon vector was derived from the fosmid, W12–3113N17 (G248P80076569; isolated from the WIBR-2 library, received from the BACPAC Resource Center, Children's Hospital Oakland, CA, USA) containing the *XPC* locus (33 kb) on chromosome 3 [13]. The fosmid was modified with HSV-1 sequences, blasticidin resistance and the marker gene for the red fluorescent protein (RFP) (pHGBsdRFP) to generate pHGXPC-Bsd-RFP (56 kb) (Fig. 1A). The second amplicon vector pα40 (55 Kb; Fig. 1B), contained 40 kb of chromosome 17 alphoid DNA (for centromere formation), the G418 resistance gene and the marker gene for the green fluorescent protein (GFP) [9,10].

2.2. Cells

The primary fibroblast cells, XPC patient derived, XP358BE, XP54BE, XP495BE, XP518BE or healthy control fibroblast lines NIH223, NIH232 [14,15], were derived from skin biopsies obtained through Institutional Review Board approved protocols at the National Cancer Institute (to KH Kraemer). Control human foreskin fibroblasts (HFF) as previously described [16] were previously isolated from an anonymous neonatal donor. For transduction, fibroblasts were grown in high glucose DMEM (ThermoFisher Scientific) supplemented with 10 % FBS (Hyclone, Logan, UT), 1 % Pen/Strep (ThermoFisher Scientific) and 8 mM HEPES (Sigma-Aldrich). For 3D skin equivalent tissue culture, fibroblasts were grown in Low Glucose DMEM (ThermoFisher Scientific) supplemented with 10 % FBS (Hyclone), 1 % Pen/Strep (ThermoFisher Scientific) and 8 mM HEPES (Sigma-Aldrich). The HT1080 cells (ECACC 85111505), Vero 2-2 (a kind gift from Rozanne M. Sandri-Goldin) and G16-9 cells, a derivative of the human glioma cell line Gli-36 [17] were grown as described previously [9,10].

2.3. Preparation of 3D skin-like tissues

The XPC patient-derived fibroblast cells tested included XP358BE, XP54BE, XP495BE, XP518BE. Together with the control fibroblast cells 223, 232, and HFF, all cells were incorporated into skin forming tissues. Each of the cells XP358BE, XP54BE, XP495BE, XP518BE cells were assessed to determine which could produce the optimal skin tissue formation [18]. Briefly, fibroblasts were mixed with Type I Collagen (Organogenesis) to a final concentration of 3×10^5 cells/ml. The collagen gels were submerged in fibroblast culture medium, where fibroblasts contracted and remodeled the collagen matrices over the course of 7 days. Then, 5×10^5 keratinocytes isolated from human neonatal foreskin were seeded in 50 μL of epidermal growth media (Organogenesis) on the surface of each collagen matrix, allowed to attach and grown for a further 5 days covered with the epidermal growth media to support keratinocyte proliferation. After 5 days, the constructs were raised to an air-liquid interface and fed from the bottom with cornification media (Organogenesis) containing 2 % serum to support full keratinocyte differentiation.

2.4. Amplicon production and transduction

HSV-1 amplicon production was as described previously [9,10]. In brief, Vero 2-2 cells were seeded in 6 well dishes 24 h before transfection, which was carried out using LipofectAMINE Plus (ThermoFisher Scientific), 2 μg of fHSVΔpacΔ270 DNA, 1.8 μg of BAC/fosmid DNA and 200 ng of plasmid pEBHICP27. After 60 h, the cells were pelleted, and the amplicon particles released by freezing and thawing and sonication. The amplicon preparations were concentrated by ultra-centrifugation onto a 25 % sucrose cushion, at 20,000 r.p.m. (4 °C) in a Beckman SW32 rotor. The titre of the amplicon preparation was estimated by transducing G16-9 cells with known volumes of suspension and counting the number of fluorescent cells per field.

Prior to transduction, the target cells were plated in complete medium in 6-well dishes at $2x10^4$ per well. On the day of the transduction, the medium was replaced with 500 μl of DMEM containing the XPCRFPBsd and/or pα40 amplicons at the MOI indicated. The wells were closed with sterile adhesive film, and spinoculated by centrifuging at 1500 rpm for 30 min. After 24 h, the efficiency of transduction was measured by evaluating the number of green/red fluorescent cells.

2.5. Growth conditions post-transduction

Following transduction, the medium was changed, and the cells were incubated for 24 h. The selection applied was either G418 250ug/ml with blasticidin 10 μg/ml, G418 only at 500 μg/ml, or blasticidin only at 10 μg/ml. To improve the survival of the primary fibroblast cells after transduction, cells were plated in Matrigel (Merck) 1:1 or 1:2 in DMEM or Collagen Type I: 500 μg/ml of collagen in 0.02 M acetic acid. The fibroblast cultures were grown in low oxygen (2 %) [19]. FGF2 (Merck) was added to the culture 24 h post-transduction at 100 ng/ml. Different plating densities were tested, including $2x10^4$ (confluent), $2x10^3$ and $1x10^3$ per 6 well dish.

2.6. FISH

Metaphase spreads were harvested from actively growing cells using standard techniques [20]. hBAC495J24, containing alpha satellite from chromosome 17 [9] and the W12–3113N17 fosmid containing the XPC locus, were labelled by nick translation using a commercial kit (Abbott Molecular) incorporating Chroma Tide™ Alexa Fluor™ 488-5-dUTP or 594-5-dUTP (Thermo Fisher Scientific) respectively. FISH on metaphase spreads was performed as described previously [9,10].

To perform FISH experiments on FFPE sections, the paraffin was removed by xylene washes. The samples were treated at in 10 mM citrate buffer pH6.0 at 95 °C for 20 min. Following protein digestion with

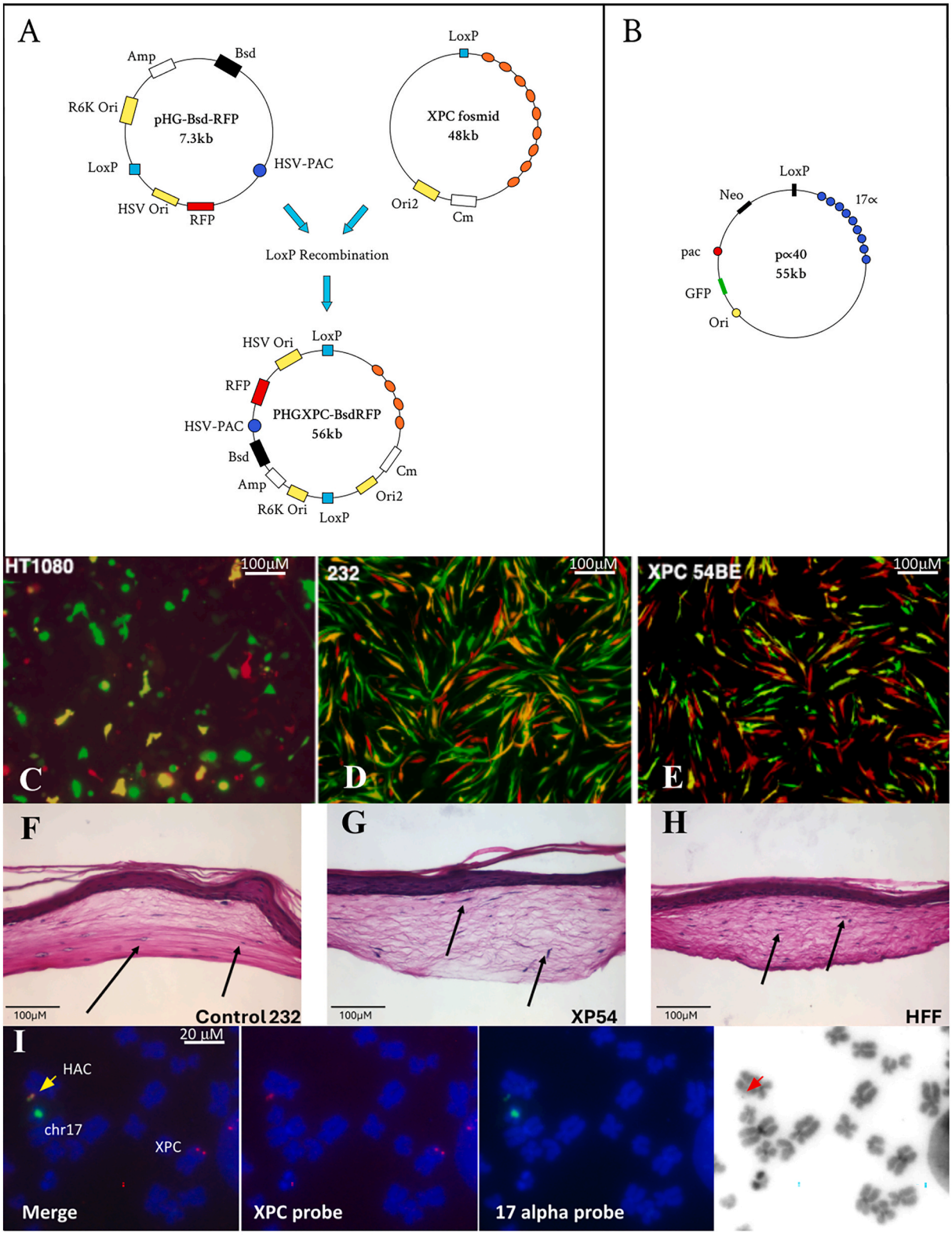

Fig. 1. Panels (A, B) Vectors used in dual transduction experiments: A XPC + RFP; B alpha satellite + GFP; Panels (C–E) GFP and RFP expression 24 h after co-transduction of pα40 and pHGXPC-Bsd-RFP in HT1080 (C), 232 (D) and XPC XP54BE (E); Panels (C–E) Skin equivalent fabrication incorporating control 232 fibroblasts (C), XPC fibroblasts (D) and foreskin fibroblasts (E). The arrows identify cells with typical fibroblast morphology. (I) FISH analysis of metaphase chromosome clones generated in HT1080 cells, with DNA probes for 17α (green), and XPC DNA (red). The leftmost panel shows a merged image with the two FISH probes, with chromosomes stained in DAPI, blue. The endogenous chromosome 17 and XPC gene locus are also identified. The HAC is labelled with a yellow arrow. The second panel shows the signal from the XPC DNA (red) probe only, the third panel shows the 17α (green) only, and the fourth panel shows the DAPI staining, inverted, to allow identification of the HAC (red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Digest All 3 (ThermoFisher Scientific), probes and cellular DNA were denatured simultaneously by incubating at 85 °C for 5 min. Hybridization was allowed to continue overnight. Post-hybridization washes were in 0.1xSSC at 60 °C. Autofluorescence was reduced with the Vector® True VIEW® Autofluorescence Quenching Kit (Vector Laboratories), following the manufacturer instructions.

Metaphase spread images were acquired with an Olympus BX-51 epifluorescence microscope equipped with a JAI CVM4+ progressive-scan 24 fps black and white fluorescence CCD camera, using the Leica Cytovision software. FISH on FFPE sections was analyzed with a Leica DM6B microscope for epifluorescence, equipped with a DFC 9000 Gt B&W fluorescence CCD camera, and operated via the Leica LASX software.

3. Results

The vectors pHGXPC-Bsd-RFP (containing the *XPC* gene locus, Fig. 1A) and pα40 (containing chromosome 17 alpha α satellite DNA, Fig. 1B) for HAC formation were prepared as HSV-1 amplicons for simultaneous transduction delivery into HT1080 and XPC deficient primary cells.

3.1. Generation of the XPC HAC in HT1080 cells

In control experiments, the viability of the vectors pHGXPC-Bsd-RFP and pα40, was initially tested in HT1080 (fibrosarcoma) cells to determine if the introduction of both vectors successfully generated an *XPC* gene containing HAC, and to monitor the HAC forming frequency following transduction. HAC formation in HT1080 cells is generally high following amplicon transduction, and therefore this cell line is a useful indicator to test new candidate HAC vectors for HAC formation and to determine the optimal transduction conditions [9,10].

The HSV-1 amplicon vectors pHGXPC-Bsd-RFP (containing the *XPC* gene locus) and pα40 (containing chromosome 17 alpha satellite DNA and the gene for G418 resistance) were co-transduced at MOI 5 into HT1080 cells. After 24 h, fluorescent green (GFP) from pα40, red (RFP) from pHGXPC-Bsd-RFP and yellow (co-localized red and green) signals were monitored. The number of cells detected for each signal indicated the transduction efficiency (Table 1, Fig. 1C–E). The efficiency for pα40 was 20%, for pHGXPC-Bsd-RFP was 3 % and for pHGXPC-Bsd-RFP plus pα40 was 17 %.

Following selection for both vectors with the addition of antibiotics, blasticidin and G418, for 7 days, stable clones grew and were isolated and expanded for further analysis. Five clones from each experiment were analyzed in further detail. Metaphase chromosome spreads were prepared from the positive cells isolated from each clone. Each clone was analyzed by FISH with probes specific for the 17α and the *XPC* DNA. The results showed that the 17α probe detected an exogenous signal from pα40 alone and from cells transduced with both pα40 and pHGXPC-Bsd-RFP. The *XPC* DNA detected exogenous signals from cell clones transduced with pHGXPC-Bsd-RFP alone and both pα40 and pHGXPC-Bsd-RFP. Both the 17α and the *XPC* DNA probes successfully co-localized and produced an autonomous yellow signal in cells transduced with pα40 and pHGXPC-Bsd-RFP. This indicated that the two vectors had recombined in HT1080 cells and generated an autonomous *XPC* gene HAC (Fig. 1I). In some clones, HAC DNA was also detected at a low frequency within the HT1080 genome. The positive results showed that simultaneous transduction of the two HSV-1 amplicons containing vectors pHGXPC-Bsd-RFP and pα40, succeeded in producing *XPC* gene HAC formation in fibrosarcoma cells.

Table 1
Transduction efficiency in HT1080 cells. Probes for 17α DNA detected pα40 and XPC DNA detected pHGXPC-Bsd-RFP.

HT1080 MOI 5	Efficiency	Clones analyzed	FISH
pα40	20 %	5	HAC
pHGXPC-Bsd-RFP	3 %	5	Integrated DNA
pα40 + pHGXPC-Bsd-RFP	17 %	5	HAC

3.2. Selection of XPC deficient primary fibroblast cells

We next proceeded to test primary human fibroblasts isolated from XPC deficient patients. Cells were analyzed to determine which would be optimal for the construction of 3D skin equivalent tissues. XP fibroblasts XP358BE, XP54BE, XP495BE, XP518BE, were compared to three control primary human fibroblast lines 223, 232, and HFF which were not deficient for XPC. Based on the ability of these fibroblasts to support the development and differentiation of the surface epithelium, XP54BE and the control 232 primary cells generated optimal tissue structure compared to the other XPC deficient cell lines (Fig. 1F–H). The optimal tissue morphology was characterized by the development of differentiated 3D skin-like tissues epithelial tissues and a dermis-like connective tissue showing typical fibroblast morphology typically found in normal skin formation *in vivo* [18]. These factors indicated that the XP54BE cell line might offer optimal fibroblast function when incorporated into 3D skin-like tissues and was therefore selected as the prime candidate for co-transduction of the pHGXPC-Bsd-RFP and pα40 amplicon vectors alongside control 232 fibroblast cells.

3.3. Co-transduction of HSV-1 amplicons into XPC 54 primary fibroblast cells

The HSV-1 amplicons vectors pα40 and pHGXPC-Bsd-RFP, were then co-transduced into the XPC patient XP54BE (deficient for XPC protein) cells and control 232 fibroblast primary cells. We set up the experiment with amplicon titres at MOI 5 and MOI 10 to determine the optimum transduction efficiency. After 24 h, fibroblast cells from both XPC XP54BE and 232 were monitored by fluorescence for GFP (green), RFP (red) and yellow cells derived from co-localization of the pα40 (GFP, green) and pHGXPC-Bsd-RFP (red) vectors (Fig. 1C–E). The scored results indicated the efficiency of co-transduction in the different cell lines (Table 2). Fig. 1C–E compared images of the transient expression in XP54BE cells with the control 232 cells at the different MOI 5 and MOI 10. The 232 and XP54BE fibroblast cells both showed a high percentage of co-localized yellow signals at MOI 5 and 10 after 24 h. The transduction frequency for XP54BE was 21 % at MOI 5 and 61 % at MOI 10. Similarly for the 232 control cells the transduction efficiency was 26 % at MOI 5 and 62 % at MOI 10 (Table 2). This indicated that a high level of transduction in the primary fibroblasts could be obtained at MOI 10. The signals were monitored daily and remained detectable in both cell lines for up to 4 days. The data suggested that the yellow signal resulting from the colocalization of signals from the genes for GFP (green), and RFP (red) resulted from expression from a HAC generated in the XPC deficient primary XP54BE fibroblasts as detected in HT1080 cells.

To generate stable clones, G418 and blasticidin was applied to both cell lines after 48 h to select for cells containing the *XPC* gene HAC, and cell growth was monitored for 14 days. After 14 days, both XP54BE and 232 cells in the presence or absence of antibiotic selection, entered senescence and no viable clones were generated.

3.4. Effect of growth conditions on cell viability in primary fibroblasts (WT and XPC deficient)

The co-transduction experiments above were repeated in XP54BE

Table 2
Transduction efficiency in XPC cells.

pα40 + pHGXPC-Bsd-RFP	Control 232 cells	XPC54BE
MOI 5	26 %	21 %
MOI 10	62 %	61 %

and control 232 cells under different growth conditions to determine if cell viability could be prolonged post transduction and if stable clones could be generated in the presence or absence of selection. Firstly, fibroblast cells were grown under hypoxic conditions in low oxygen (2 %), to prolong the lifespan of the primary cultures [19]. Secondly, to mimic a physiological environment, the fibroblast cells were plated and cultured onto Matrigel or collagen which coated the wells of the dish. Thirdly, FGF2 was added to the culture media. Lastly, fibroblast cells were plated at different densities prior to transduction including $2x10^4$, $2x10^3$, and $1x10^3$ since high confluency is known to affect fibroblast capacity of division [21]. None of the above conditions tested allowed the XPC cells to remain viable and generate stable clones 14 days post transduction.

3.5. HAC analysis in XPC 3D skin-like tissues

We determined if growing the cells in our 3D tissue culture system supported cell survival following transduction. XP54BE and 232 control cells expressing fluorescent yellow signals (co-localized from both GFP and RFP) after 24 h were incorporated into 3D skin-like tissues and were grown for 12 days as described above. Formalin-fixed, paraffin-embedded tissues that were stained using hematoxylin and eosin demonstrated normal tissue organization and morphology. Tissues were hybridized by FISH with 17α and *XPC* DNA probes to determine if signals from the 17α DNA and the *XPC* gene remained. While signals for the endogenous chromosomes 17, and XPC loci were visible, no colocalizing signal, corresponding to a HAC was identified. However, the data indicated that the skin tissue formation following transduction with HSV-1 amplicons was viable. It is feasible that the HAC frequency was too low for detection by FISH, or that the HAC were not maintained in the cells during the two weeks needed for 3D skin tissue development.

4. Discussion

HSV-1 amplicon vectors containing the *XPC* gene and essential HAC forming sequences were successfully co-introduced simultaneously into wild type (HT1080 fibrosarcoma) and DNA repair deficient (XPC patient) fibroblasts (XP54BE cells) using the dual (co-infection) transduction method [11]. An *XPC* gene expressing HAC was generated in stable HT1080 cell lines. In XP54BE deficient patient primary fibroblasts, co-localized fluorescent (yellow) signals from the GFP (green) and RFP (red) markers were detected. The result confirmed that genes were transiently expressed from an HSV-1 *XPC* HAC vector. After two weeks in the presence or absence of antibiotic selection, the positive XP54BE cells from the transductions became senescent and fluorescent signals were no longer detected. Compared to HT1080 fibrosarcoma cells, no stable clones from the transduced XPC deficient primary skin cells were generated due to the onset of senescence in the primary cells.

Positive fluorescent XPC XP54BE cells detected at 24 h post transduction were developed into 3D skin-like tissues to observe tissue formation. Tissues were mounted onto microscope slides for analysis by FISH with *XPC*, 17α and vector DNA probes. No exogenous *XPC* expression from a HAC was detected in the 3D tissue sections. However, XP54BE fibroblasts persisted in the tissues as seen by their support of epithelial development and compared to the control HFF fibroblasts. It is possible that HAC transduction led to the premature senescence of transduced cells in 2D culture, while non-transduced cells persisted to support 3D tissue development. Alternatively, the HSV-1 transduction may have reduced the fitness of the transduced cells, allowing the non-transduced cells to increase in number.

The premature senescence seen in transduced XPC patient primary fibroblasts (but not in HT1080 cells) was investigated further, but the cause was not identified in the present study. None of the growth conditions tested including growth in low oxygen tension, on Matrigel or collagen, with basic FGF2, or plating cells at different densities increased the survival of primary transduced cells. It is possible that the senescence in the primary fibroblasts was triggered by the HSV-1 amplicon transduction, and further experiments are required to identify the factors which led to cell death post transduction.

It is possible that the innate immune response was activated in the primary XPC XP54BE cells following infection with HSV-1 amplicons and triggered senescence. Different cells initiate different responses to infection by HSV-1 amplicons. Tsitoura et al. [22] observed a mild antiviral HSV-1 response in HFF, but not in lung fibroblasts. We observed no antiviral response in human embryonic stem cells which produced stable clones containing HSV-1 gene expressing HACs after transduction [8]. The amplicon system we used was constructed to eliminate viral genes and prevent contamination and/or amplification of amplicon sequences post transduction [23,24]. However, some cells may be more sensitive to stress than others and even if a low level of contaminating particles resulting from sequences within the vectors remains, then an inflammatory stress response may be initiated together with gene silencing. Cell death may occur by different mechanisms in cells depending on the type and level of IFN and NF KB cytokine response and the ability of the introduced particles to evade it [25].

A systematic approach is required to investigate the challenges of determining why some cells and not others produce an antiviral response to HSV-1 amplicons and the factors that contribute to cell senescence following transduction in primary fibroblasts. Also, further work is required to improve the process of isolating and transfecting skin stem cells that will persist in 3D skin-like tissues, thereby an alternative to using primary fibroblast cells. This will be important for developing a new generation of *XPC* HAC HSV-1 amplicons vectors for *ex vivo* gene therapy in XPC patients. Sustaining long-term transgene expression in XPC deficient primary fibroblasts will require studies to further elucidate how HSV-1 HAC vector transduction can be successfully applied for *XPC* gene therapy.

CRediT authorship contribution statement

Daniela Moralli: Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Avi Smith:** Investigation, Data curation. **Jonathan Garlick:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kenneth H. Kraemer:** Writing – review & editing, Validation, Resources, Methodology, Data curation. **Zoia L. Monaco:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

I certify that the information given is true and complete to the best of my knowledge. I declare there are no financial or competing sources of interest.

Acknowledgements

Thank you to Anthony F.L. Monaco for modifications to Fig. 1. This work was supported by the NIH NIAMS grant 1 R21AR066938-01; Wellcome Trust Core Award Grant, 203141/Z/16/Z. Informed consent was provided to Kenneth Kraemer 99C0099L/357239, and supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

References

[1] J.J. Digiovanna, K.H. Kraemer, Shining a light on xeroderma pigmentosum, J. Invest. Dermatol. 132 (2012) 785–796.
[2] S.G. Khan, V. Muniz-Medina, T. Shahlavi, C.C. Baker, H. Inui, T. Ueda, S. Emmert, T.D. Schneider, K.H. Kraemer, The human XPC DNA repair gene: arrangement,

splice information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function, Nucleic Acids Res. 30 (2002) 3264–3631.
[3] K.-S. Oh, K. Imoto, S. Emmert, D. Tamura, J.J. DiGiovanna, K. Kraemer, Nucleotide excision repair proteins rapidly accumulate but fail to persist in human XP-E (DDB2 mutant) cells, Photochem. Photobiol. 87 (2011) 729–733.
[4] E. Renaud, L. Miccoli, N. Zacal, D.S. Biard, C.T. Craescu, A.J. Rainbow, J.F. Angulo, Differential contribution of XPC, RAD23A, RAD23B and CENTRIN 2 to the UV-response in human cells, DNA Repair 10 (2011) 835–847.
[5] L. Zeng, X. Quilliet, O. Chevallier-Lagente, E. Eveno, A. Sarasin, M. Mezzina, Retrovirus mediated gene transfer corrects DNA repair deficit of Xeroderma Pigmentosum cells of complementation groups A, B, and C, Gene Ther. 4 (1997) 1077–1084.
[6] A.H. Ryu, W.L. Eckalbar, A. Kreimer, N. Yosef, N. Ahituv, Use antibiotics in cell culture with caution: genome-wide identification of antibiotic-induced changes in gene expression and regulation, Sci. Rep. 7 (2017) 7533.
[7] A. Dupuy, A. Sarasin, DNA damage and gene therapy of Xeroderma Pigmentosum, a human DNA repair-deficient disease, Mutat. Res. 776 (2014) 2–8.
[8] A. Terunuma, J. Ye, S. Emmert, S.G. Khan, K.H. Kraemer, J.C. Vogel, Ultraviolet light selection assay to optimize oligonucleotide correction of mutations in endogenous xeroderma pigmentosum genes, Gene Ther. 11 (2004) 1729–1734.
[9] D. Moralli, K.M. Simpson, R. Wade-Martins, Z.L. Monaco, A novel human artificial chromosome gene expression system using Herpes Simplex Virus Type1 vectors, EMBO Rep. 7 (2006) 911–918.
[10] M.A. Mandegar, D. Moralli, S. Khoja, S. Cowley, D.Y. Chan, M. Yusuf, S. Mukherjee, M.P. Blundell, E.V. Volpi, A.J. Thrasher, W. James, Z.L. Monaco, Functional human artificial chromosomes are generated and stably maintained in human embryonic stem cells, Hum. Mol. Genet. 20 (2011) 2905–2913.
[11] D. Moralli, Z.L. Monaco, Gene expressing human artificial chromosome vectors: advantages and challenges for gene therapy, Exp. Cell Res. 390 (2020). PMCID: PMC7166075.
[12] D.Y.L. Chan, D. Moralli, L. Wheatley, J. Jankowska, Z.L. Monaco, Multigene human artificial chromosome vector delivery with Herpes Simplex Virus 1 amplicons, Exp. Cell Res. 153 (2020) 88.
[13] K. Osoegawa, A.G. Mammoser, C. Wu, E. Frengen, C. Zeng, J.J. Catanese, P.J. de Jong, A bacterial artificial chromosome library for sequencing the complete human genome, Genome Res. 11 (2001) 483–496.
[14] C. Kuschal, S.G. Khan, B. Enk, J.J. DiGiovanna, K.H. Kraemer, Readthrough of stop codons by use of aminoglycosides in cells from xeroderma pigmentosum group C patients, Exp. Dermatol. 24 (2015) 296–297.
[15] S.G. Khan, K.S. Oh, T. Shahlavi, T. Ueda, D.B. Busch, H. Inui, S. Emmert, K. Imoto, V. Muniz-Medina, C.C. Baker, J.J. DiGiovanna, D. Schmidt, A. Khadavi, A. Metin, E. Gozukara, H. Slor, A. Sarasin, K.H. Kraemer, Reduced XPC DNA repair gene mRNA levels in clinically normal parents of xeroderma pigmentosum patients, Carcinogenesis 27 (2006) 84–94.
[16] T. Compton, An immortalized human fibroblast cell line is permissive for human cytomegalovirus infection, J. Virol. 67 (1993) 3644–3648.
[17] M. Sena-Esteves, J.A. Hampl, S.M. Camp, X.O. Breakefield, Generation of stable retrovirus packaging cell lines after transduction with herpes simplex virus hybrid amplicon vectors, J. Gene Med. 4 (2002) 229–239.
[18] M.W. Carlson, A. Alt-Holland, C. Egles, J.A. Garlick, Three-dimensional tissue models of normal and diseased skin, Curr. Protoc. Cell Biol. (2008) 1–23. Chapter 19: Unit 19.9.
[19] A.K. Balin, L. Pratt, Oxygen modulates the growth of skin fibroblasts, In Vitro Cell. Dev. Biol. Anim. 38 (2002) 305–310.
[20] D. Moralli, M. Yusuf, M.A. Mandegar, S. Khoja, Z.L. Monaco, E.V. Volpi, An improved technique for chromosomal analysis of human ES and iPS cells, Stem Cell Rev Rep 7 (2011) 471–477.
[21] A.I. McClatchey, A.S. Yap, Contact inhibition (of proliferation) redux, Curr. Opin. Cell Biol. 24 (2012) 685–694.
[22] E. Tsitoura, J. Thomas, D. Cuchet, K. Thoinet, P. Mavromara, A.L. Epstein, Infection with herpes simplex type 1-based amplicon vectors results in an IRF3/7-dependent, TLR-independent activation of the innate antiviral response in primary human fibroblasts, J. Gen. Virol. 90 (2009) 2209–2220.
[23] A.L. Epstein, HSV-1 derived amplicon vectors: recent technological improvements and remaining difficulties – a Review, Mem. Inst. Oswaldo Cruz 104 (2009) 399–410.
[24] E. Tsitoura, A.L. Epstein, Constitutive and innate responses in cells infected by HSV-1 derived amplicon vectors, Open Virol. J. 4 (2010) 96–102.
[25] K.A. Milora, S.L. Miller, J.C. Sanmiguel, L.E. Jensen, Interleukin-1a released from HSV-1 infected keratinocytes acts as a functional alarmin in the skin, Nat. Commun. 5 (2014) 5230.