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Brief Definitive Report

Cancer Cell Detective Story: Identifying the Unique Molecular Biomarkers of Cancer for Administering Appropriate Treatment Therapies

Mary-Kate Daniel

Cancer was the leading cause of nearly 10 million deaths worldwide in 2020. At least one third of such cancer cases are preventable. To halt the harmful and uncontrollable division of abnormal cells in cancer, a wide range of treatments in the form of radiation, chemotherapy, surgery, and medications are utilised to target dangerous growth through inhibiting cell proliferation and preventing metastasis. However, the two primary forms of such treatments, known as conventional and targeted therapy, possess advantageous effects for cancer diagnosed patients whilst having the potential to induce harmful complications that hinder the efficiency of multiple biological processes. Although conventional therapy efficiently damages telomeric DNA of cancerous cells to halt tumorigenesis and the harmful proliferation of abnormal cells, the cancer treatment causes irreparable destruction to healthy cells and tissue. Targeted therapy is utilised to prevent such indiscriminate damage through specifically attacking the cancerous cells. However, cancer cells may become resistant to such targeted therapy, resulting in untreated tumours or malignant growths. The following investigation aimed to identify the unique molecular profiles of three different cancer cell lines through detecting the human papillomavirus (HPV) strain 18 by a polymerase chain reaction to confirm the role of the adenomatous polyposis coli (APC) in disrupting the Wnt signalling pathway that induces a tumorigenic phenotype. The presence of APC was identified through the restriction fragment length polymorphism (RFLP). Through utilising immunocytochemistry (ICC) it was discovered that the cell line positive for Alternative Lengthening of Telomeres (ALT) presented longer telomeres than that of the two telomerase-positive cell lines.

Introduction

Cancer can manifest in many different forms amongst different bodily organs whilst possessing varying degrees of severity. Accordingly, recognising the major hallmarks as well as the distinctive genomic features that characterise specific cancers will enable individualized treatment plans based on the molecular features of each patient's condition whilst advancing cancer biomarker discovery. Conventional chemotherapeutic drugs are administered to specific cancer diagnosed patients to disrupt the protein and RNA synthesis that contribute to neoplastic cell proliferation, inducing mitotic catastrophe whilst damaging telomeric DNA (Amjad MT, Chidharla A, Kasi A. 2022). However, conventional therapy also targets DNA replication within normal host cells, a characteristic that is absent in targeted therapy in which specific molecular proteins contributing to tumorigenesis are exploited to inhibit cancer growth whilst preventing harm to regular cells (Baudino T. 2015).

The type of treatment therapy chosen for each patient is dependent on the stage and severity of cancer growth, factors that are regulated by genetic components such as the tumour suppressor gene Adenomatous polyposis coli (APC). Germline and somatic mutations within the APC gene produce truncated gene sequences that disrupt the Wnt signalling pathway responsible for mediating cell polarity, migration, organogenesis and immune cell renewal, commonly resulting in familial adenomatous polyposis (FAP) that frequently leads to colorectal cancers (CRC) (Zhang and Shay, 2017).

Accordingly, the sexually transmitted disease human papillomavirus (HPV) has the potential to infect the genomic DNA of the host cell nucleus, disrupting the oncoproteins E6 and E7 to induce their degradation of the tumour suppressor protein p53, a cell responsible for inducing apoptosis during the G1 phase of the cell cycle to repair DNA damage (Rai et al., 2016). Chromosomal instability due to telomere shortening, a protective cap at the end of DNA sequences that are regulated by the ribonucleic protein telomerase, is a critical component of cancer initiation. Alternative Lengthening of Telomeres (also known as "ALT") is a telomerase-independent mechanism by which cancer cells avoid the degradation of telomeres (Jafri et al., 2016). Cancer cells avoid telomere attrition typically induced by repeated DNA replication through the Alternative Lengthening of Telomeres (ALT), in which cancer cells bypass apoptosis through homologous recombination mechanism of DNA replication, enabling de novo telomere synthesis that produces proteins with long heterogenous telomeres (Jafri et al., 2016).

The following investigation aims to discover cancer hallmarks that efficiently reveal the appropriate targeted therapy by identifying the presence of human papillomavirus (HPV) strain 18 DNA in three separate cancer cell lines through utilising polymerase chain reaction (PCR). Furthermore, the PCR

experiment accompanied by the Restriction fragment length polymorphism technique to identify mutations within the APC gene sequence. Lastly, the investigation aims to observe the differences in telomeric foci by immunocytochemistry.

RESULTS AND DISCUSSION

Practical 1 - Results & Discussion

In practical 1, DNA extraction and quantification from the three Eppendorf tubes containing cell pellets from separate cancer cell lines was performed by utilizing the commercial QIAamp DNA Mini Kit to amplify HPV18 and APC by PCR, resulting in one positive cancer cell line for HPV18 (Figure B). Therefore, confirming the role of the APC gene in disrupting the Wnt signaling pathway in HPV-infected cervical cancer cells in response to cooperation of E6 and E7 gene expression in inducing a tumorigenic phenotype (Ayala-Calvillo et al., 2017). From utilising PCR to discover the expression of the two primary oncogenes for progressing cervical cancer at the hands of the HPV infection, the potential to develop a targeted treatment therapy that disrupts the Wnt signalling pathway in a strategy that parallels the tumour-suppressor action of the APC gene arises (Pal and Kundu, 2020). Recognising E6 and E7 oncogenes as significant hallmarks for cervical cancer advance the development of efficient treatment therapies, ranging from genomic editing technology, vaccines and T-cell regulated immunotherapy that may target the harmful proliferation of cancer cells. Furthermore, E6 has a significant role in stimulating the tumour suppressor protein p53 via a trimeric complex that includes the protein expression regular E6-AP, arresting the cell cycle to induce cell apoptosis and DNA sequence repair. The E7 oncogene accompanied E6 in preventing cancer cell proliferation through binding to the 'pocket domain' of the Retinoblastoma protein (Rb) protein to initiate tumour suppressor function through inhibiting cell cycle progression (Yim and Park, 2005).

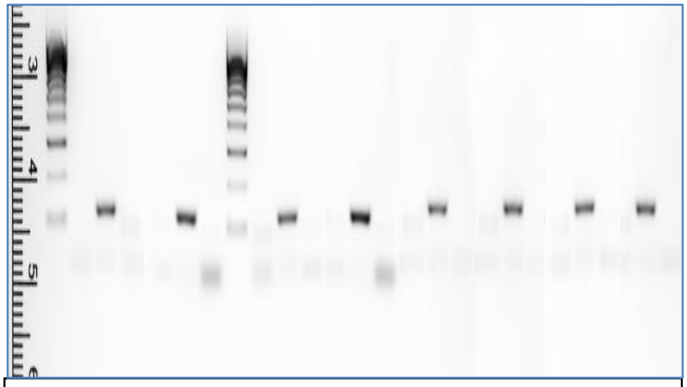

Figure A – Gel image of human papillomavirus (HPV) polymerase chain reaction (PCR) to detect the presence of HPV strain 18 within one cell line.

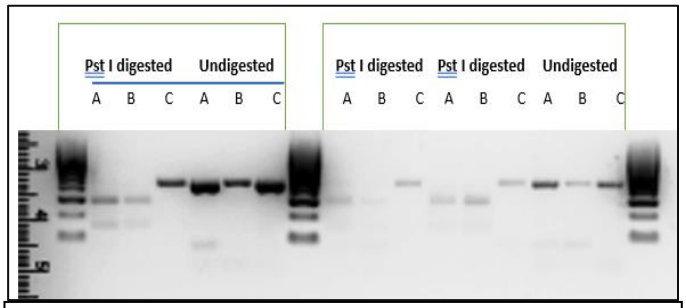

Figure B – Gel image of human papillomavirus (HPV) polymerase chain reaction (PCR) to detect the presence of HPV strain 18 within one cell line.

Practical 2 – Results and Discussion

In practical 2, ICC is performed by using an antibody against the shelterin protein TRF2 to observe the telomere phenotype of the three different cell lines. It was found that two of the cell lines are telomerase positive with homogenous fluorescence (figure B) whereas one cell line is positive for ALT with long telomeres. Therefore, the telomerase activity present in the two cell lines is an indicator of high cancer cell proliferation that manifest in short telomeres whereas the cell line positive for ALT confirms the recombination-based replication mechanism that cancer cells adopt to maintain telomere length and induce neoplastic formation (Skvortsov, D,A et al. 2011).

From this finding, the ALT pathway in which cancer cells utilise to maintain telomere length serves as a biomarker or hallmark for prognostic purposes and the advancement of treatment therapies that target the harmful proliferating cells that favour this pathway. Furthermore, further research into the cause of molecular attraction between specific cancer cells and the features of the ALT pathway in order to determine the unique biological features of cancer tissues that are particularly triggered by the alternate path of telomere lengthening (Nersisyan et al., 2021).

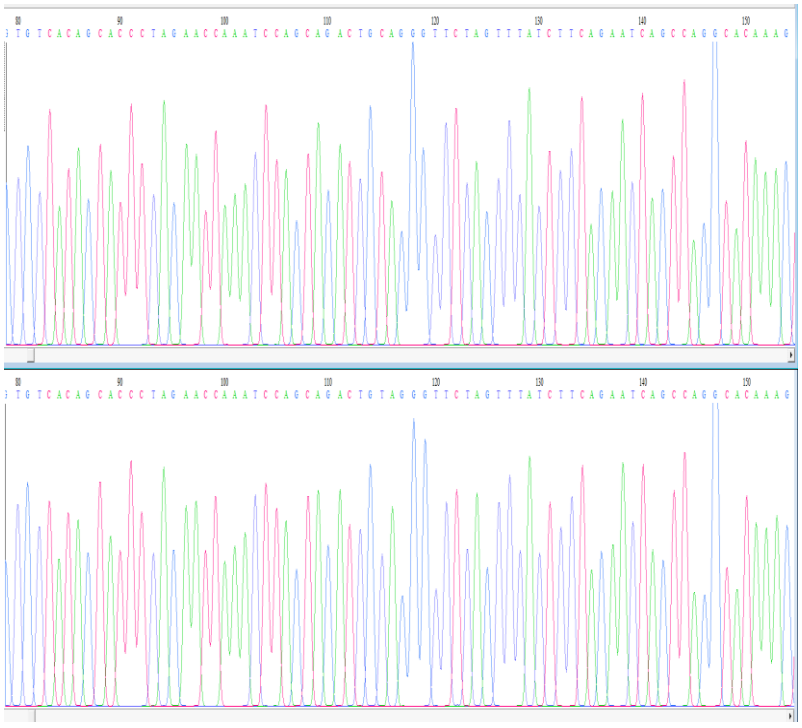

Figure C – Representation of Sanger Sequencing results revealing the nucleotide sequence of the known oncogene APC DNA sequence utilising restriction fragment length polymorphism. The detected mutation within the DNA sequence indicates the potential for familial adenomatous polyposis (FAP), a condition preceding the development of CRC.

MATERIALS AND METHODS

Practical 1 - Materials & Methods

Practical 1 aims to amplify HPV18 and APC by PCR to enable RFLP analysis for APC. Accordingly, the experiment begins with DNA extraction by utilising the QIAamp DNA Mini kit, in which 3 Eppendorf tubes containing cell pellets from different cancer cell lines were incubated with 20uL proteinase K and 200 uL Buffer AL at 56°C for 10 minutes prior to being pulse-vortexed with 200uL of ethanol for 15 seconds. The mixtures were applied to the QIAamp spin column to be utilised in fast spin-column procedures between the range of 8000-14,000rpm at varying times. Thus, isolating DNA to be quantified using a spectrophotometer as it binds to the QIAamp silica-gel membrane whilst contaminants leave the medium. PCR amplification follows DNA extraction to visualise the cancer cell line taken from a cervical tumour that is positive for HPV by utilising a PCR mix containing HPV18 primer solution (10uM) (Set 1) as well as a PCR mix containing APC primer solution (10uM) (Set2). Practical 1 ended with a RFLP test to detect the hotspot mutation in APC in which a restriction mix containing nuclease free water, 10x 3.1 Buffer and restriction enzyme (Pstl) is mixed in a 1.5mL tube on ice, accompanied by 40uL of PCR master mix being added to 10uL of Set 2 in a 1.5mL tube. The reaction was spun in a microcentrifuge prior to incubating in a water bath at 37°C for 30 minutes.

Practical 2 – Materials and Methods

Practical 2 began with performing the Immunocytochemistry (ICC) technique in which an antibody against the shelterin protein telomeric repeat-binding factor 2 (TRF2) to produce microscopic images of the telomere phenotype. The experiment began by setting up a humidity chamber with 200uL ABDIL overlayed slides that were incubated at room temperature for 1 hour. A 1.500 dilution of the TRF2 antibody was added to the 200uL ABDIL overlayed slides before being incubated at room temperature for 1 hour. A 1:400 dilution of rabbit Alexa Fluor 488 secondary antibody was added to the slides to be incubated at room temperature for 30 minutes prior to being rinsed in PBS and accompanied by two drops of Prolong gold antifade solution. The fluorescent dye revealed that two of the cell lines fixed with 10% formalin are telomerase positive, whereas one cell line is ALT positive as represented by long heterogenous telomeres.

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