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A Study of Radiation-Induced Telomere Instability Using Multiplex Ligation-Dependent Probe Amplification (MLPA)

Alan Chant[1], Ahmad Chaudary[2], Christina M. Kraemer-Chant[3*]

[1]Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT, USA
[2]Department of Public and Allied Health, Bowling Green State University, Bowling Green, OH, USA
[3]Department of Chemistry, Saint Michael's College, Colchester, VT, USA
Email: *cchant@smcvt.edu

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Abstract

The integrity of the chromosomes for two WIL2-derived lymphoblastoid cell lines (TK6 and WTK1) in the presence and absence of ionizing radiation was analyzed by Multiplex Ligation-Dependent Probe Amplification (MLPA). The TK6 cell line has the native p53 tumor-suppressor gene, whereas WTK1 cells contain a p53 mutation. Each cell line was isolated pre- and post-irradiation (2 and 3 Gy) and analyzed by MLPA. The impact of irradiation on these two cell lines was investigated using probes that target specific regions on chromosomes associated with subtelomeric regions. Results indicate that WTK1 and TK6 are impacted differently after irradiation, and that each cell line presents its own unique MLPA profile. The most notable differences are the appearance of a number of probes in the post-irradiated MLPA profile that are not present in the controls, and two unique probe signals only seen in WTK1 cells. These results build on our previous studies that indicate how different human cell lines can be affected by radiation in significantly different ways depending on the presence or absence of wild type p53.

Keywords

Ionizing Radiation, Multiplex Ligation-Dependent Probe Amplification (MLPA), p53 Tumor Suppressor, Telomere Instability

1. Introduction

p53, also known as tumor protein P53 or cellular tumor antigen p53, is a critical cell-cycle check point protein that regulates the G1 phase of the cell cycle. It is also directly responsible for maintaining genomic DNA stability during the

DNA damage repair stage, and it coordinates the repair of damaged DNA and the removal of DNA lesions before the cell enters S-phase. The p53 gene has been identified as one of the most frequently mutated genes in human cancers [1] [2] [3] [4], but it remains unclear how far-reaching the effects of these mutations are on cell integrity. The p53 regulatory protein is crucial in the cell's response to DNA damage as it plays a direct role in the DNA repair pathways, affecting the activities of a number of diverse regulatory proteins that collectively control the early stages of the cell cycle [5]-[19]. Mutation or the removal of p53 could lead to genomic instability, resulting in numerous chromosomal issues [20]-[26]. Apoptosis has also been reported to be affected by the presence of p53 mutants, as mutated cell lines show a decreased frequency of apoptotic death. This decrease could in turn lead to the accumulation of gene mutations and eventual progression of tumorigenesis [27] [28] [29] [30].

In this study, the effects of ionizing radiation on two closely related WIL2-derived lymphoblastoid cell lines (TK6, or thymidine kinase 6, and WTK1) were analyzed. The WTK1 cell line is a TP53-knockout mutant; the p53 gene in the WTK1 cell line has a substitution at codon 237, which leads to a mutation from methionine to isoleucine in the p53 protein. This cell line will overexpress only mutant p53 protein. Therefore, no wild-type p53 protein is present in the WTK1 cells. The TK6 cell line is a thymidine kinase heterozygote cell line that is wild-type for p53 [31] [32] [33] [34]. Previous studies on the effects of ionizing radiation on these two cells lines indicate that the WTK1 cell line is more resistant to radiation-induced killing than the TK6 cell line. There is significantly less apoptosis in WTK1 when compared to TK6, and mutability was also shown to be drastically different, with TK6 exhibiting a 10-fold decrease when compared to WTK1 [35] [36].

Our lab is interested in utilizing Multiplex Ligation-Dependent Probe Amplification (MLPA) to study the effects of ionizing radiation on specific regions of the chromosomes, focusing on the differences between the WTK1 and TK6 cell lines before and after irradiation at various levels of intensity. MLPA has become a preferred method for detecting deletions and amplifications in human genes [37] [38] [39] [40]. This study specifically focused on deletions or duplications in subtelomeric regions of genomic DNA. The probe set utilized—P070 (MRC Holland)—was selected as it can be utilized to analyze deletions and duplications in subtelomeric regions in genomic DNA. As per the manufacturer (https://www.mrcholland.com/product/P070/1147), aberrant copy numbers of subtelomeric regions are a frequent cause of intellectual disabilities, developmental delays, and/or congenital abnormalities that lack distinct syndrome features.

2. Materials and Methods

2.1. Cell Lines and Cell Culturing

Cells used were the thymidine kinase heterozygote cell line TK6 and the TP53

mutant cell line WTK1. Both cell lines were cultured and maintained as previously described [41].

2.2. Irradiation of Cell Lines and Preparation for MLPA Analysis

The two lymphoblastoid cell lines (TK6 and WTK1) were treated with ionizing radiation prepared for MLP analysis as described previously [41].

2.3. Preparation of Genomic DNA for MLPA Analysis

Total Genomic DNA was prepared as described previously [41].

2.4. PCR Amplification

Amplification of the ligated probes chosen to study a region known to be associated with congenital abnormalities was carried out by polymerase chain reaction (PCR) using the SALSA MLPA Probemix P070 Subtelomeres Mix 2B. This mix is designed to probe for 41 subtelomeric regions of interest plus an extra probe for the subtelomeric regions on the short q-arm of the 5 acrocentric chromosomes. PCR amplification and fragment analysis was performed as previously described [41].

2.5. Statistical Analysis

Statistical analysis was carried out as described previously [41].

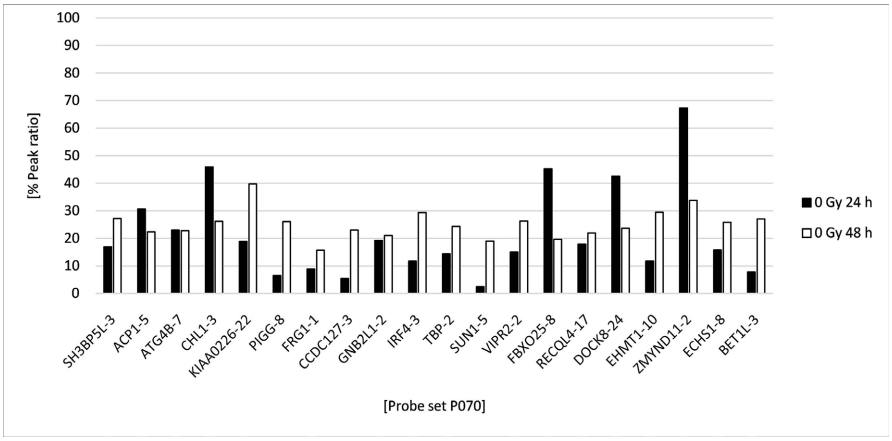
3. Results

This study specifically focused on using MLPA to investigate chromosomal copy number imbalances associated with telomere instability.

3.1. MLPA Analysis—TK6 Results

Figure 1 shows the data for 0 Gy exposures to irradiation after 24 and 48 h incubation for the TK6 cell line as a function of probe signal intensity. This serves as the control for this cell line. The percent peak ratio for 25.0% of the probes decreases to varying extents, between 1.1% and 72.7%, between the 24 and 48 h incubation samples, potentially due to cellular degradation. The other probes (75.0%) increase in signal intensity between 9.4% and 1283.3%. By comparing the data in Figure 1 with data gathered under similar conditions post-irradiation, any changes that might have occurred as a result of ionizing radiation can be identified.

Figure 2 shows the MLPA data for 2 and 3 Gy exposures to irradiation after 24 and 48 h incubation for the same (TK6) cell line as a function of probe signal intensity. Most probes (77.6%) in this probe set for 2 Gy irradiation show an increase in percent peak ratio between 24 and 48 h incubation, ranging from 21.5% to 3012.0%. The remainder—KDM5A-23 and VAMP7-8—show a decrease between the two time points, with KDM5A-23 showing a decrease of 27.4% and a VAMP7-8 showing a decrease of 45.6%. Fewer probes (37.9%) in



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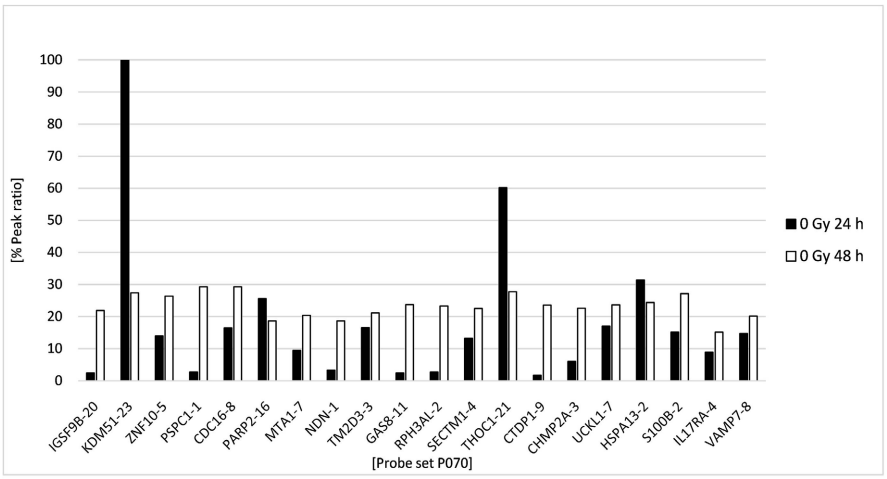
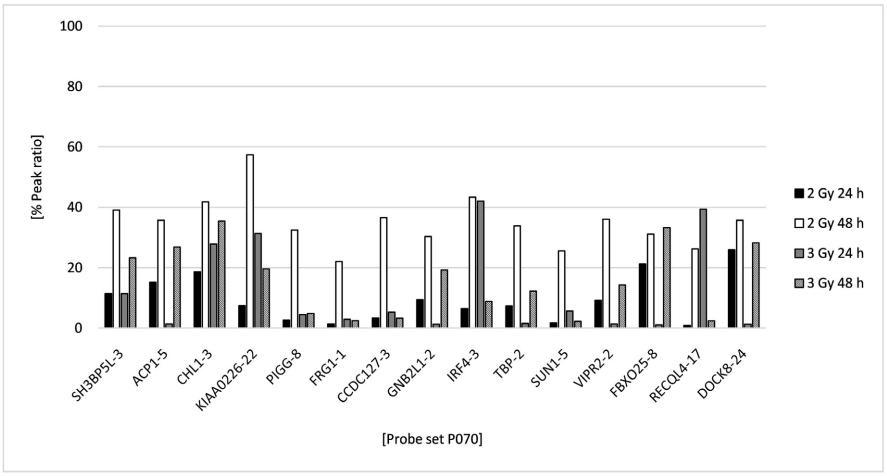


(b)

Figure 1. The data after 24 (black bars) and 48 (white bars) h incubation for 0 Gr exposure to irradiation for the TK6 cell line as a function of probe signal intensity. This is the control for this cell line. Each set of bars represents different probes in the P070 probe set.



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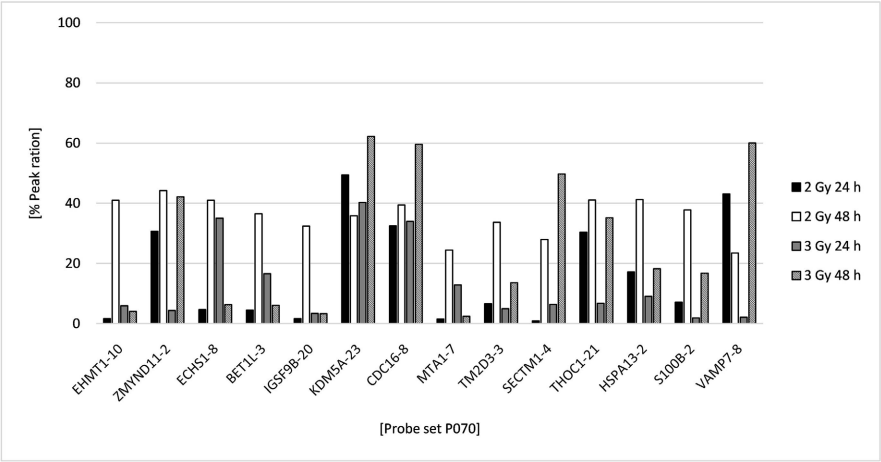


(b)

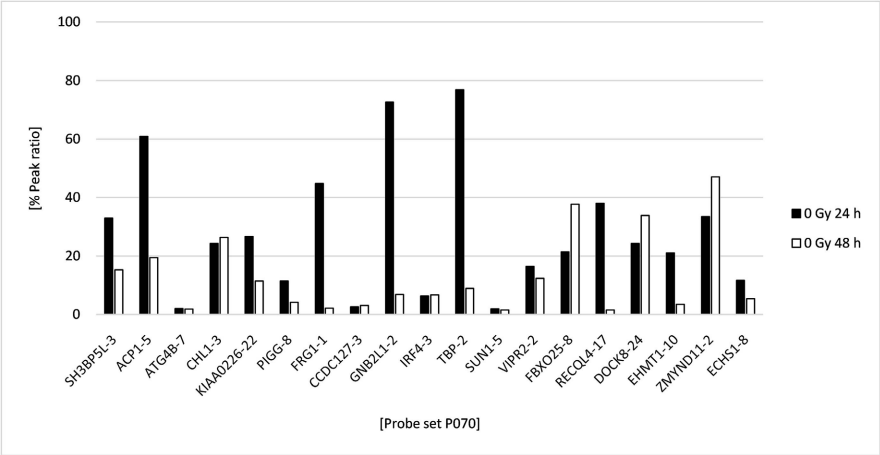
Figure 2. The data after 24 (black and gray bars) and 48 (white and hashed bars) h incubation for 2 Gy (black and white bars) and 3 Gy (grey and hashed bars) exposure to irradiation for the TK6 cell line as a function of probe signal intensity. Each set of bars represents different probes in the P070 probe set.

this probe set for 3 Gy irradiation show an increase in percent peak ratio between 24 and 48 h incubation, ranging from 9.9% to 2882.8%. More probes for the 3 Gy irradiation show a decrease as compared to the 2 Gy, with a minimum decrease of 1.9% and a maximum decrease of 93.9%. Interestingly, some of the probes experiencing some of the largest decreases (or increases) in percent peak ratio for the 2 Gy radiation between 24 and 48 h experienced the largest increase (or decrease) for the 3 Gy radiation with the same timepoints. In other words, the 2 Gy change was, for some of the probe sets, significantly different than that observed for the 3 Gy change. For example, VAMP7-8, which was the probe that experienced the largest decrease in percent peak ratio for 2 Gy, experienced the second largest increase in percent peak ratio for 3 Gy. It is unclear what drives this opposing reaction to the 2 and 3 Gy irradiation for certain probes.

Finally, the post-irradiation data indicates copy number imbalances in the TK6 cell line for eleven probes (ATG4B-7, ZNF10-5, PSPC1-1, PARP2-16, NDN-1, GAS8-11, RPH3AL-2, CTDP1-9, CHMP2A-3, UCKL1-7, and IL17RA-4). These eleven probes were present only in the control group; they were not present in either post-irradiation conditions (2 or 3 Gy).

3.2. MLPA Analysis—WTK1 Results

Figure 3 shows the data for 0 Gy exposure to irradiation after 24 and 48 h incubation for the WTK1 cell line as a function of probe signal intensity. This serves as the control for this cell line. The percent peak ratio for 34.2% of the probes increases to varying extents, between 2.9% and 289.5%, between the 24 and 48 h incubation samples, potentially due to cellular degradation. Decreases ranging from 8.8% to 95.9% occur in the majority (65.8%) of the probes. This indicates that the mutant cell line seems to be more affected by this degradation than the



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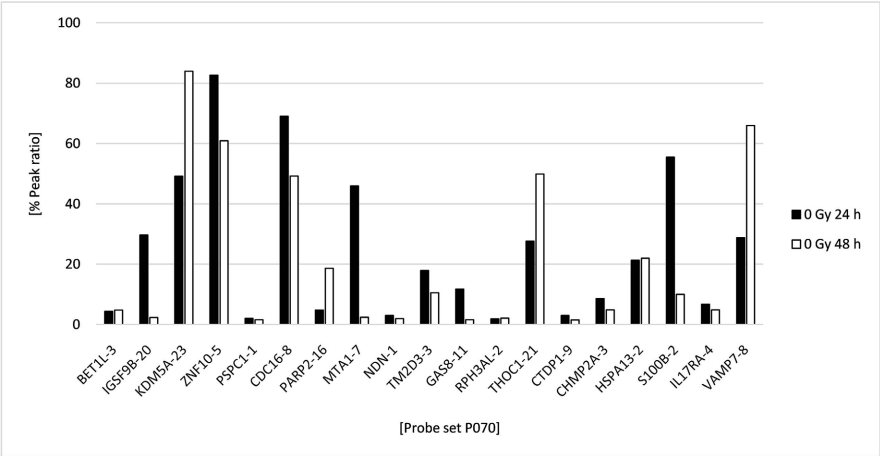


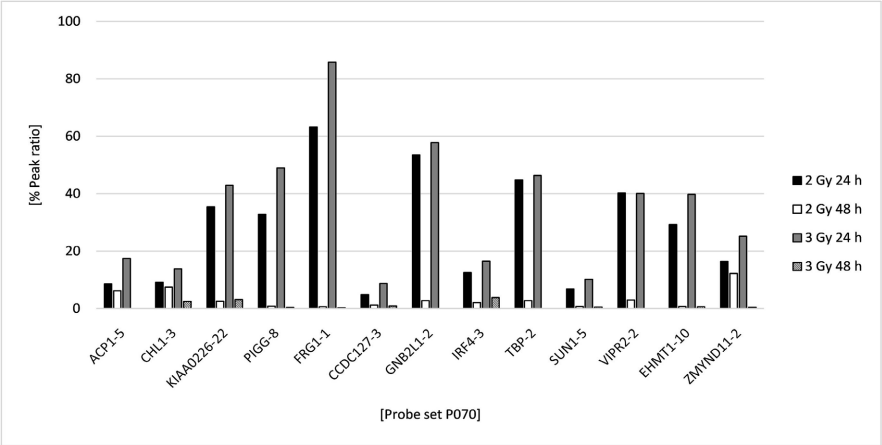
(b)

Figure 3. The data after 24 (black bars) and 48 (white bars) h incubation for 0 Gy exposure to irradiation for the WTK1 cell line as a function of probe signal intensity. Each set of bars represents different probes in the P070 probe set.

wild-type TK6 cell line. This result has been observed before by our group when analyzing these same two cell lines for radiation-induced instability using alternative probe sets.

Two probe sets—SEKTM1-4 and UCKL1-7—are present in the control results for the TK6 cell line yet are not present in the control results for the WTK1 cell line. One of those (UCKL1-7) also shows a loss in probe enhancement for the TK6 cell line pre- and post-irradiation. The implications of these results are not yet known.

By again comparing the control data with data gathered under similar conditions post-irradiation, changes in the mutant cell line that may have occurred due to ionizing radiation can be identified much as they were for the wild-type cell line. **Figure 4** shows the MLPA data for 2 Gy and 3 Gy exposures to irradiation after 24 and 48 h incubation for the WTK1 cell line as a function of probe signal intensity. All but 2 probes (92.0%) in this probe set for 2 Gy irradiation



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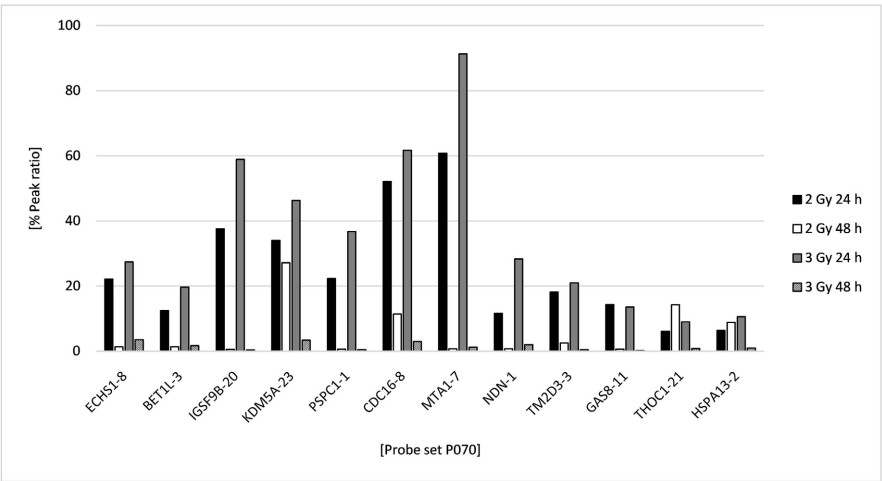

(b)

Figure 4. The data after 24 (black and gray bars) and 48 (white and hashed bars) h incubation for 2 Gy (black and white bars) and 3 Gy (grey and hashed bars) exposure to irradiation for the WTK1 cell line as a function of probe signal intensity. Each set of bars represents different probes in the P070 probe set.

show a decrease in percent peak ratio between 24 and 48 h incubation, ranging from 18.6% to 99.0%. Only two probe sets show an increase (THOC1-21 (134.9%) and HSPA13-2 (40.1%)). No probes in this probe set for 3 Gy irradiation show an increase in percent peak ratio between 24 and 48 h incubation; the decreases range from 76.5% to 87.1%. Finally, the post-irradiation data does indicate copy number imbalances in the WTK1 cell line, which is evident in the loss of probe enhancement for thirteen probes (SH3BP5L-3, ATG4B-7, FBXO25-8, RECQL4-17, DOCK8-24, ZNF10-5, PARP2-16, RPH3AL-2, CTDP1-9, CHMP2A-3, S100B-2, IL17RA-4, and VAMP7-8).

3.3. MLPA Analysis—Comparison of TK6 and WTK1 Results

SECTM1-4 and UCKL1-7 are found in the control for the TK6 cell line but are not found in the WTK1 cell line control. Three probes (PSPC1-1, NDN-1, and

Table 1. Probes described in Section 3.3. Information is taken from genecards.org (accessed 4 Mar. 2024).

Gene	Gene name	Role or function	Pathology
PSPC1	Paraspeckle component 1	Controls gene expression *via* an RNA nuclear retention mechanism	Inborne genetic disease
GAS8	Growth arrest-specific protein8	Putative tumor suppressor protein	Cillary dyskinesia
UCKL1	Uridine-cytidine kinase 1-like 1	Catalyzes the phosphorylation of uridine to uridine monophosphate	Inborne genetic disorder
SH3BP5L	SH3 binding domain protein 5-like	Involved in intracellular signal transduction andnegative regulation of kinase activity	Inborne genetic disease
S100B	S100 calcium binding protein B	Regulation of cell cycle progression andcell differentiation	Syringoma; neurofibroma
VAMP7	Vesicle-associated membrane protein	Transmembrane protein	Tetanus; gonadal dysgenesis
SECTM1	Secreted and transmembrane 1	May be involved in thymocyte signaling	Ulceroglandular tularemia; corneal dystrophy; familial apolipoprotein C-Ii deficiency; epithelial and subepithelial dystrophy
NDN-1	Necdin, MAGE family member	Growth suppressor that facilitates the entry of the cell into cell cycle arrest; also interacts with p53 to inhibit cell growth	Prader-Willi syndrome
FBXO25-8	F-Box protein 25	Substrate-recognition component of the SCF (SKP1-CUL1-F-box protein)-type E3 ubiquitin ligase complex	A chromosomal aberration involving FBXO25 is a cause of X-linked intellectual disability (XLID)
RECQL4-17	RecQ-like helicase	May play a role in the repair of DNA damaged by ultraviolet light or other mutagens; magnesium-dependent ATP-dependent DNA-helicase activity	Recon Progeroid syndrome; hereditary breast ovarian cancer syndrome
DOCK8-24	Dedicator of cytokinesis 8	Activates small GTPase CDC42 by exchanging bound GDP for free GTP; required for interstitial dendritic cell (DC) migration; required for CD4(+) T-cell migration; involved in NK cell cytotoxicity	Hyper-Ige syndrome 2, autosomal recessive; combined immunodeficiency due to Dock 8 deficiency; severe combined immunodeficiency; autosomal dominant non-syndromic intellectual disability

GAS8-11) that are found in both control and post-irradiated WTK1 cell lines are found in the TK6 cell line control but are not found in the post-irradiation samples. Six probes (SH3BP5L-3, FBXO25-8, RECQL4-17, DOCK8-24, S100B-2, and VAMP7-8) that are found in both control and post-irradiated TK6 cell lines are

found in the WTK1 cell line control but are not found in the post-irradiation samples. No probes were located post-irradiation that were not present in the controls for both cell lines. It appears from these results that radiation-induced telomere instability was more prevalent in the mutant cell line than in the wild type, which was to be expected.

4. Discussion

This study was performed in order to understand the effects of ionizing radiation on the copy-number variations of the subtelomeric regions of chromosomes in two closely related cell lines: TK6 (the wild type) and WTK1 (the p53 mutant). The focus was to understand whether there was any connection between aberrant copy numbers and the presence or absence of p53.

WTK1 contains the mutant p53 (M237I) at the thymidine kinase (tk) locus [42] and has been shown to play a major role in maintaining genetic stability. Among other findings, p53 mutants have been shown to have loss of DNA binding function that prevents them from carrying out their regulatory role [43]. Mutant p53 appears able to engage in aberrant interactions with other cellular factors, which typically results in gain-of-function phenotypes [44] [45] [46].

The application of Multiplex Ligation-Dependent Probe Amplification (MLPA) on TK6 and WTK1 has provided a wealth of information into the impact on chromosomal instability after being subjected to ionizing radiation. Our results show that there are unique alterations of probe enhancement for both TK6 and WTK1 cells. These results showed a number of notable gene deletions that are involved in general gene regulation, signal transduction, tumor suppression, regulation of the cell cycle and posttranslational modifications. The impact of abnormal gene expression, specifically loss of expression, could potentially lead to a unique set of pathologies that have been linked to developmental delay and congenital abnormalities (see for example **Table 1**, which lists the 11 unique probes described in Section 3.3 above).

Finally, we have demonstrated that, while WTK1 and TK6 share genes that have been impacted in the same way by the irradiation, each cell line presents its own unique response profile. However, both cell types share changes in important cellular functions that can lead to cancer and disease. Each cell line seems equally impacted in the areas of regulation, signal transduction, tumor suppression, regulation of the cell cycle and posttranslational modifications. These results hold significant importance in the study of the effects of ionizing radiation in human cells, and how different cell lines can be affected in significantly different ways depending on the presence of wild type p53.

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Conflicts of Interest

The authors declare no commercial or financial conflict of interest.

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