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Radiation-Induced Chromosome Instability in WTK1 and TK6 Human Lymphoblastoid Cells

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Abstract

The effects of ionizing radiation on single nucleotide polymorphism (SNP) copy number variations between TK6 and WTK1 cell lines are described herein. Specifically, the integrity of the chromosomes for two WIL2-derived lymphoblastoid cell lines (TK6 and WTK1) was analyzed in the presence and absence of ionizing radiation. WTK1 cells contain a p53 mutation, whereas the TK6 cell line has the native p53 tumor-suppressor gene. Each cell line was isolated post-irradiation for SNP analysis, which showed significant, genome-wide impacts on both cell lines; for the mutant WTK1 sample, there were a total of 48 gene deletions and no gene amplifications, whereas for the wild-type TK6 sample, there were 217 gene deletions and 9 gene amplifications. It appears that both cell lines are affected in the areas of cell-cycle control, but that other affected areas differ significantly between the two.

Keywords

Ionizing Radiation, Single Nucleotide Polymorphism, p53 Tumor Suppressor

1. Introduction

p53 is a critical cell-cycle check-point protein that regulates the G1 phase of the cell cycle and is directly responsible for maintaining genomic DNA stability during the DNA damage repair stage. It is known to coordinate the repair of damaged DNA and the removal of DNA lesions before the cell enters S-phase of the cell cycle. Therefore, it is easy to imagine that any problems that might occur

during the G1 cell-cycle arrest would impact normal repair to the damaged DNA that occurs prior to entering S-phase. The p53 gene has been studied extensively and has been identified as one of the most frequently mutated genes in human cancers [1] [2] [3] [4]. The question of how far-reaching the effects of these mutations are on cell integrity is still being studied. The p53 regulatory protein is crucial in the cell's response to DNA damage and 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]. This would further lead to genomic instability, resulting in abnormal numbers of chromosomes, gene amplifications, chromosomal rearrangement, deletions, insertions, accumulation of double-strand breaks, and gene amplifications [20]-[26]. Controlled cell death (apoptosis) has also been reported to be affected by p53 mutated proteins, as cell lines with these mutations have been shown to have a reduced frequency of apoptotic death, which could in turn lead to the accumulation of gene mutations and progression of tumorigenesis [27] [28] [29] [30].

For this study, we analyzed the effects of ionizing radiation on two closely related WIL2-derived lymphoblastoid cell lines (TK6, or thymidine kinase 6, and WTK1). The WTK1 cell line is a TP53-knockout mutant derived from the WIL2 parent cell line. Specifically, the p53 gene in the WTK1 cell line has a substitution at codon 237, which leads to a mutation in the p53 protein from methionine to isoleucine. This cell line will only overexpress mutant p53 protein; no wild type p53 will be present. This cell line has been used so frequently in previous research on the effects of ionizing radiation that it can be considered the "gold standard" for these types of studies. The TK6 cell line, in contrast, is also derived from the WIL2 parent cell line but 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 the two cells lines show that the WTK1 cell line is more resistant to radiation-induced killing and that there is significantly less apoptosis in WTK1 when compared to TK6. Mutability was also shown to be drastically different, with TK6 exhibiting a 10-fold decrease when compared to WTK1 [35] [36].

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 each cultured in a 75 cm^2 tissue culture flask at 37˚C with 8% CO_2 and maintained at a cell concentration between 2×10^5 and 1×10^6 cells/mL. The culture medium consisted of RPMI 1640 and 10% v/v heat-inactivated horse serum (Gibco®, a ThermoFisher Scientific Company, Waltham, MA, USA).

2.2. Irradiation of Cell Lines and Preparation for SNP Analysis

The two lymphoblastoid cell lines (TK6 and WTK1) were treated with ionizing radiation (3 Gy vs. 0 Gy control) using a ^{137}Cs biological irradiator (Gamma-

cell-1000 Unit). Each cell line was irradiated 0.3 cm from the surface with a dose rate of 6.41 Gy/min for 28 seconds to provide a total of 3 Gy exposure. After irradiation, cells were incubated for 48 hours at 37˚C under 8% CO_2, followed by isolation for SNP analysis. Whole genomic DNA was isolated from both cell lines using a DNeasy Blood and Tissue Kit (Qiagen). Approximately 5 million cultured cells (WTK1 or TK) were centrifuged separately for 5 minutes at 300 ×g. After centrifugation, 200 µL of PBS (50 mM potassium phosphate, 150 mM NaCl, pH 7.2) was used to resuspend the pellet. Once all cells were in free suspension, 20 µL of Proteinase K (600 mAU/mL) and 4 µL of RNase A (100 mg/mL) were added, and the cells were incubated at room temperature for 2 minutes. After the incubation period, 200 µL of AL buffer (Qiagen) was added, the suspension was mixed by vortexing, and the cells were incubated again for 10 minutes at 56˚C. The sample tube was left to cool to room temperature before 200 µL 95% molecular grade ethanol was added, and the sample was vortexed until it was thoroughly mixed. The sample was then placed in a DNeasy spin column and centrifuged at 6000 ×g for 1 minute. The flowthrough and collection tube were discarded and the DNeasy spin column was placed in a new tube. 500 µL of AW1 buffer (Qiagen) was added to the tube and centrifuged at 6000 ×g for 1 minute. The DNeasy spin column containing the DNA was placed in a new collection tube and 500 µL of AW2 was added and centrifuged at 20,000 ×g for 1 minute. The DNeasy column was then put into a clean tube and 200 µL of AE buffer (Qiagen) was added to the membrane and left to incubate at room temperature for 1 minute. The sample was then eluted by centrifuging at 6000 ×g for 1 minute.

2.3. Analysis of SNPs

50 µg of whole genomic DNA from each cell type was then analyzed using a 256 k STY microarray chip, and analysis of single nucleotide polymorphisms (SNPs) was carried out using an Affymetrix GeneChip Human Mapping 250 K Sty. Partek Genomic Suite's Copy Number analysis (CN) and Allele-specific copy number (ASCN) workflows were utilized for the two samples using the paired analysis option.

3. Results

3.1. SNP Analysis—Integration of ASCN and CN Results and P-Values

To confirm allele deletions and detect copy neutral genomic events, ASCN results were integrated with CN results. Table 1 and Table 2 show the raw data set from the SNP analysis. Statistical analysis shows the P-values for all the probes listed to be of a high degree of confidence.

3.2. SNP Analysis—Specifics of TK6 and WTK1 Results

Interestingly, no two genes from the TK6 and WTK1 samples were affected by

Table 1. Integrated ASCN results with CN results for TK6.

Chr	Cytoband	Start	End	Length (bps)	Length (Kb)	Modification	No. probes	Probe density	Mean copy number	P-value	Overlapping RefSeq genes
1	1p32.2	58018411	58102775	84364	84	Amplification	13	6489.54	3.04943	0.00180806	Intron of DAB1 (--−)
2	2p21	47182709	47259414	76705	77	Deletion	11	6973.18	1.17658	0.00445066	Contained within TTC7A (+)
2	2p25.1	9784529	9857416	72887	73	Deletion	10	7288.7	1.18714	0.000653079	None
2	2q14.2	119893616	120103405	209789	210	Deletion	26	8068.81	1.35894	0.00581587	C1QL2 (--−), STEAP3 (+), region overlaps with 20.78% of C2orf76 (--−)
3	3p26.1	6394113	6490483	96370	96	Deletion	14	6883.57	1.30877	0.0468727	None
4	4q23	100164895	100319304	154409	154	Deletion	12	12867.4	1.37538	0.0193024	ADH1A (--−), ADH1B (--−), ADH1C (--−), region overlaps with 37.3% of LOC100507053 (+)
5	5q31.1	131646277	131969143	322866	323	Deletion	27	11958	1.37316	0.00514616	C5orf56 (+), IL5 (--−), IRF1 (--−), LOC553103 (--−), MIR3936 (--−), SLC22A5 (+), region overlaps with 10.4% of SLC22A4 (+), 23.7% of RAD50 (+)
5	5q33.1	150982753	151040778	58025	58	Deletion	10	5802.5	1.18605	0.00123703	Region overlaps with 0.2% of SPARC (--−)
6	6p12.1	53226579	53361010	134431	134	Deletion	13	10340.8	1.2829	0.0113972	None
6	6p25.1	6598746	6665270	66524	67	Deletion	14	4751.71	1.27237	0.0131841	Region overlaps with 36.6% of LY86--−AS1 (--−), 84.9% of LY86 (+)
7	7p13	44783038	45237489	454451	454	Deletion	39	11652.6	1.46163	0.0140781	CCM2 (+), H2AFV (--−), MIR4657 (--−), MYO1G (--−), NACAD (--−), PPIA (+), PURB (--−), RAMP3 (+), SNHG15 (--−), SNORA5A (--−), SNORA5B (--−), SNORA5C (--−), SNORA9 (--−), TBRG4 (--−), ZMIZ2 (+)
7	7q35	145066826	145882546	815720	816	Amplification	27	30211.9	2.91072	2.99E--−05	Region overlaps with 8.5% of CNTNAP2 (+)
10	10q22.1	72660683	73858777	1198094	1198	Deletion	184	6511.38	1.58835	0.00311729	C10orf105 (--−), C10orf54 (--−), CDH23 (+), CHST3 (+), LOC102723377 (--−), MIR7152 (+), PSAP (--−), SLC29A3 (+), SPOCK2 (--−), UNC5B (+), UNC5B--−AS1 (--−), region overlaps with 0.2% of ASCC1 (--−)
11	11p15.1	19576970	19620319	43349	43	Deletion	10	4334.9	1.19839	0.000673858	MIR4486 (+), intron of NAV2 (+)
13	13q14.11	40883885	40954431	70546	71	Deletion	10	7054.6	1.21126	0.0203006	Region overlaps with 47.0% of LINC00598 (--−)
16	16p13.3	86671	2372535	2285864	2286	Deletion	131	17449.3	1.55843	0.00107079	ARHGDIG (+), AXIN1 (--−), BAIAP3 (+), BRICD5 (--−), C16orf13 (--−), C16orf91 (--−), C1QTNF8 (--−), CACNA1H (+), CAPN15 (+), CASKIN1 (--−), CCDC154 (--−), CCDC78 (--−), CHTF18 (+), CLCN7 (--−), CRAMP1L (+), DECR2 (+), DNASE1L2 (+), E4F1 (+), ECI1 (--−), EME2 (+), FAHD1 (+), FAM173A (+), FAM195A (+), FBXL16 (--−), GFER (+), GNG13 (--−), GNPTG (+), HAGH (--−), HAGHL (+), HBA1 (+), HBA2 (+), HBM (+), HBQ1 (+), HBZ (+), HN1L (+), HS3ST6 (--−), IFT140 (--−), IGFALS (--−, ITFG3 (+), JMJD8 (--−), LINC00235 (--−), LINC00254 (--−), LMF1 (--−),

Continued

											LMF1-−AS1 (+), LOC100134368 (+), LUC7L (-−), MAPK8IP3 (+), MEIOB (-−), METRN (+), MIR1225 (-−), MIR3176 (+), MIR3177 (+), MIR3180-−5 (-−), MIR3677 (+), MIR4516 (+), MIR4717 (+), MIR5587 (+), MIR6511B1 (-−), MIR6511B2 (-−), MIR662 (+), MIR940 (+), MLST8 (+), MPG (+), MRPL28 (-−), MRPS34 (-−), MSLN (+), MSRB1 (-−), NARFL (-−), NDUFB10 (+), NHLRC4 (+), NME3 (-−), NME4 (+), NOXO1 (-−), NPRL3 (-−), NPW (+), NTHL1 (-−), NUBP2 (+), PDIA2 (+), PGP (-−), PIGQ (+), PKD1 (-−), POLR3K (-−), PRR25 (+), PRR35 (+), PTX4 (-−), RAB11FIP3 (+), RAB26 (+), RAB40C (+), RGS11 (-−), RHBDF1 (-−), RHBDL1 (+), RHOT2 (+), RNF151 (+), RNPS1 (-−), RPL3L (-−), RPS2 (-−), RPUSD1 (-−), SLC9A3R2 (+), SNHG9 (+), SNORA10 (-−), SNORA64 (-−), SNORA78 (+), SNORD60 (-−), SNRNP25 (+), SOX8 (+), SPSB3 (-−), SSTR5 (+), SSTR5-−AS1 (-−), STUB1 (+), SYNGR3 (+), TBL3 (+), TELO2 (+), TMEM204 (+), TMEM8A (-−), TPSAB1 (+), TPSB2 (-−), TPSD1 (+), TPSG1 (-−), TRAF7 (+), TSC2 (+), TSR3 (-−), UBE2I (+), UNKL (-−), WDR24 (-−), WDR90 (+), WFIKKN1 (+), ZNF598 (-−), region overlaps with 2.0% of ABCA3 (-−)
16	16q22.1	68713776	68766540	52764	53	Deletion	10	5276.4	1.18734	0.0108732	Region overlaps with 36.4% of CDH3 (+)
16	16q24.3	88856351	90156776	1300425	1300	Deletion	95	13688.7	1.53951	0.00253683	ACSF3 (+), AFG3L1P (+), ANKRD11 (-−), APRT (-−), CBFA2T3 (-−), CDH15 (+), CDK10 (+), CDT1 (+), CENPBD1 (-−), CHMP1A (-−), CPNE7 (+), DBNDD1 (-−), DEF8 (+), DPEP1 (+), FANCA (-−), GALNS (-−), GAS8 (+), GAS8-−AS1 (-−), LINC00304 (+), LOC100129697 (+), LOC100287036 (+), LOC101927817 (+), LOC400558 (+), MC1R (+), PABPN1L (-−), PRDM7 (-−), RPL13 (+), SLC22A31 (-−), SNORD68 (+), SPATA2L (-−), SPATA33 (+), SPG7 (+), SPIRE2 (+), TCF25 (+), TRAPPC2L (+), TUBB3 (+), URAHP (-−), VPS9D1 (-−), VPS9D1-−AS1 (+), ZNF276 (+), ZNF778 (+)
17	17q21.31	44120955	44267657	146702	147	Amplification	27	5433.41	2.66323	0.0147722	Contained within KANSL1 (-−)
17	17q21.33-−17q22	50041539	52770545	2729006	2729	Amplification	175	15594.3	2.42975	0.0147755	C17orf112 (+), KIF2B (+), region overlaps with 7.1% of CA10 (-−), 7.2% of CA10 (-−), 7.2% of CA10 (-−)
20	20q12	40524911	40574279	49368	49	Amplification	10	4936.8	3.22544	0.000732926	None

Continued

20	20q13.32	58180686	58302046	121360	121	Amplification	10	12136	3.15384	0.00611637	LOC100506384 (---), intron of PHACTR3 (+), region overlaps with 4.8% of PHACTR3 (+), 41.5% of PHACTR3 (+), 81.1% of PHACTR3 (+)
21	21q22.2	39949569	40032072	82503	83	Deletion	10	8250.3	1.18693	0.0143156	Contained within ERG (---), region overlaps with 8.9% of ERG (---)
21	21q22.3	43461765	43534781	73016	73	Deletion	10	7301.6	0.937121	0.0138014	UMODL1-–AS1 (---), region overlaps with 59.4% of UMODL1 (+), 70.8% of UMODL1 (+)
22	22q12.3	34634252	34899238	264986	265	Amplification	34	7793.71	2.61709	0.00171403	None

UV irradiation in the same way. Specifically, no one deletion or amplification was seen on the same gene of a chromosome in both samples. The genes of some chromosomes did have deletions in both samples, but for separate, distinct genes on each chromosome. In addition, most of the modifications caused by UV irradiation were gene deletions, whereas amplifications are lesser in frequency. These deletions and amplifications in specific chromosomes are uniquely linked to each cell type. The only chromosomes that showed no change were chromosomes 8, 14, 18 and 19.

When comparing the data for TK6 (Table 1) and WTK1 (Table 2) cell samples, there were 13 deletions in chromosome 1 for WTK1 and a single amplification of one gene (DAB1) in the TK6 sample. Both chromosomes 2 and 3 showed 4 deletions each, all of which were located in WTK1; TK6 showed no changes for either chromosome. Chromosome 4 showed 5 deletions for WTK1 and 4 deletions for TK6, but again, these were for different genes. TK6 showed a high incidence of deletions in chromosome 5 - 9 deletions were identified on this chromosome, as compared to 4 in the WTK1 sample. Chromosome 6 showed 2 deletions for TK6 and 4 deletions for WTK1. TK6 showed a high number of deletions again in chromosome 7 - 13 deletions were identified on this chromosome, with a single amplification for one gene (specifically SNTNAP2). WTK6 showed only 1 deletion and no amplifications for this chromosome (chromosome 7).

Only 1 gene was affected on chromosome 9 for WTK1 (on gene GBBR2), which was a deletion. There was no effect seen on chromosome 9 for the TK6 sample. For chromosome 10, there were 16 total deletions, 3 in the WTK1 sample and 13 in the TK6 sample. Chromosome 11 showed 5 deletions; 3 were in the TK6 sample, and 2 were in the WTK1 sample. Again, there were no overlaps between the two samples. Chromosome 12 had 2 deletions only, and both were in the WTK1 sample.

Chromosome 13 had 2 deletions, 1 in each sample. Chromosome 16 had a significant number of deletions (168 in all); interestingly, all these deletions were in the TK6 sample. WTK1 remained unchanged. Chromosome 17 had 2 deletions (all in WTK1) and 4 amplifications (all in TK6), and chromosome 20 had 1

Table 2. Integrated ASCN results with CN results for WTK1.

Chr	Cytoband	Start	Length (bps)	Modification	No. probes	Probe density	Mean copy number	P-value	Overlapping RefSeq genes
1	1p13.2	113149267	334135	Deletion	10	33413.5	1.15	0.0265239	AKR7A2P1 (+), CAPZA1 (+), FAM19A3 (+), LINC01356 (---), MOV10 (+), PPM1J (---), RHOC (---), overlaps with 3.8% of ST7L (---), 8.7% of SLC16A1 (---)
1	1p32.3	55715510	51613	Deletion	10	5161.3	1.15	0.0367101	None
1	1p34.3	37798637	102638	Deletion	13	7895.23	1.13	0.0182391	None
1	1p36.31	7026742	116873	Deletion	15	7791.53	1.08	0.00344254	Intron of CAMTA1 (+)
1	1q25.3	183887132	80445	Deletion	10	8044.5	1.13	0.0335717	Overlaps with 13.1% of RGL1 (+), 77.8% of COLGALT2 (---), 85.5% of COLGALT2 (---)
1	1q42.3	235738664	66894	Deletion	10	6689.4	1.12	0.0261786	Contained within GNG4 (---), intron of MIR5096 (+)
3	3p21.2	50613135	75050	Deletion	11	6822.73	1.08	0.0362439	CISH (---), MAPKAPK3 (+), overlaps with 12.4% of HEMK1 (+)
3	3p26.1---3p25.3	8673792	92304	Deletion	15	6153.6	1.10	0.0152087	Overlaps with 13.8% of SSUH2 (---), overlaps with 21.6% of SSUH2 (---)
4	4p15.33	14137469	96728	Deletion	14	6909.14	1.24	0.044563	Overlaps with 4.4% of LINC01085 (+)
4	4p16.1	6278847	93121	Deletion	15	6208.07	1.09	0.00116679	Overlaps with 28.1% of WFS1 (+), 53.3% of PPP2R2C (---)
4	4p16.1	7195569	215250	Deletion	36	5979.17	1.42	0.0296499	MIR4798 (+), contained within SORCS2 (+)
4	4q31.21	142272873	75935	Deletion	10	7593.5	1.12	0.031538	None
5	5q31.3	141202169	87032	Deletion	10	8703.2	1.11	0.0426613	LOC729080 (---), PCDH1 (---)
5	5q34	167609806	65536	Deletion	12	5461.33	1.16	0.0457192	CTB---178M22.2 (---), contained within TENM2 (+)
6	6p21.33	30879434	64559	Deletion	11	5869	1.12	0.0269568	DPCR1 (+), SFTA2 (---), VARS2 (+), overlaps with 3.8% of GTF2H4 (+)
6	6p22.3	22409457	107794	Deletion	12	8982.83	1.03	0.0162862	None
7	7q34	141967486	76479	Deletion	11	6952.64	1.17	0.0127401	TRY2P (---)
9	9q22.33	101270040	25344	Deletion	11	2304	1.04	0.0017989	Intron of GABBR2 (---)
10	10q21.2	61551896	92406	Deletion	10	9240.6	1.15	0.025666	Contained within CCDC6 (---)
10	10q24.2	100002312	87903	Deletion	16	5493.94	1.20	0.0347213	LOXL4 (---), overlaps with 2.7% of R3HCC1L (+)
10	10q25.3	115707335	28808	Deletion	13	2216	1.18	0.0195345	None
10	10q26.13	125132158	62373	Deletion	13	4797.92	1.04	0.0102152	None
11	11q23.3	120669928	92134	Deletion	14	6581	1.12	0.00354705	Contained within GRIK4 (+)

Continued

11	11q23.3	119599042	89466	Deletion	18	4970.33	1.22	0.0038485	LOC102724301 (+), overlaps with 0.4% of PVRL1 (--–)
12	12q13.11	48015131	111819	Deletion	12	9318.25	1.07	0.016288	ENDOU (--–), RPAP3 (--–)
13	13q12.12	24611705	44375	Deletion	10	4437.5	1.00	0.0351841	Intron of SPATA13 (+)
15	15q12	25853693	66972	Deletion	11	6088.36	1.18	0.0299143	None
15	15q25.3	87956746	68507	Deletion	10	6850.7	1.05	0.0299816	None
17	17p13.3	2745992	94868	Deletion	10	9486.8	1.07	0.0462259	Contained within RAP1GAP2 (+)
17	17q24.2	64942795	82532	Deletion	10	8253.2	1.11	0.00412681	Overlaps with 77.9% of CACNG4 (+)
20	20q13.13	49051633	94734	Deletion	13	7287.23	1.16	0.0270251	Overlaps with 20.6% of PTPN1 (+)
21	21q22.3	43428563	50173	Deletion	10	5017.3	0.99	0.0120412	ZNF295--–AS1 (+), overlaps with 3.9% of ZBTB21 (--–)

deletion for WTK1 (on gene PTPN1) but 3 amplifications for TK6. Finally, chromosome 21 had 5 gene deletions, 2 for WTK1 and 3 for TK6.

3.3. Identification of Genes Impacted by Irradiation in TK6 and WTK1

As shown in Table 3, out of the 18 genes listed for TK6, the most genes impacted are involved in gene regulation (PURB, E4F, POLRK, ZNF276, ZNF778, ERG) and cell cycle control (GFER, NME3, NME4, SOX8, CDK10, CDT1, TCF25). It is also significant to note that there are four genes involved in the DNA repair process. Finally, GAS8, which codes for a tumor suppressor protein, was also impacted.

Table 4 shows that, out of the 12 genes listed for WTK1, the ones most impacted are involved in tumor suppression (PPM1J, ST7L, CISH, CCDC6) and cell-cycle control (MAPAPK3, R3CC1L, PPP2R2C, ENDOU). The group of genes next most affected is involved in gene regulation (MOV10, CAMTA1, ZBTB 21). Only one gene was noted to be involved in the DNA repair process (GTF 2H4).

3.4. Summary

For the mutant WTK1 sample, there were a total of 48 gene deletions and no gene amplifications, whereas for the wild-type TK6 sample, there were 217 gene deletions and 9 gene amplifications. Besides the fact that none of these gene modifications overlapped between the two samples, these results are interesting in that the only sample showing amplifications is the wild-type sample, which also showed the largest number of gene deletions. To appreciate the differences between these two sets of data, we generated a shared segment karyogram, which shows a direct comparison of regions for deletions and amplification in the TK6 and WTK1 cells after irradiation (Figure 1).

Table 3. TK6 genes that were affected by ionizing radiation, their function/role, and pathology associated with aberrant gene expression.

Affected Gene	Function/role	Pathology
RAD 50	Double-stranded repair protein	Nijmegen Breakage Syndrome-Like Disorder and Hereditary Breast Ovarian Cancer Syndrome
PURB	Single-stranded DNA binding protein (DNA replication and transcription)	Myelodysplastic Syndrome
E4F	Transcription factor 1 (p53 related pathways)	Encephalopathy, Neonatal Severe, Due To Mecp2 Mutations and Renal Artery Obstruction
GFER	Growth factor, augnentor of liver regeneration (maintenance of mitochondrian genomes and the cell division cyle)	Myopathy, Mitochondrial Progressive, with Congenital Cataract and Developmental Delay and Mitochondrial Disease
MPG	N-methyladenine glycosylase (base excision repair)	Geotrichosis
NME3	Nucleoside diphosphate kinase (involved in the apoptotyic process)	Lipase Deficiency, Combined and Heinz Body Anemias
NME4	Nucleoside diphosphate kinase 3 (implicated in pro-apoptotic signaling)	Unknown
NTHL1	Nth-like DNA glycosylase 1	Familial Adenomatous Polyposis 3 and Bap1 Tumor Predisposition Syndrome
POLR3K	RNA Polymerase III subunit K (catayzes the transcription of DNA into RNA)	Leukodystrophy, Hypomyelinating, 21, and Leukodystrophy
SOX8	SRY-Box transcription factor 8 (involved in embryonic development and cell fate)	Alpha Thalassemia-Intellectual Disability Syndrome Type 1 and Peripheral Demyelinating Neuropathy, Central Dysmyelination, Waardenburg Syndrome, and Hirschsprung Disease
TELO2	Telomere maintenace 2 (functions in the S-phase check-point of the cell cycle and DNA repair)	You-Hoover-Fong Syndrome and Nail-Patella Syndrome
CDK10	Cyclin dependent kinase 10 (has role in cellular proliferation in the G2-M phase)	Al Kaissi Syndrome and Toe Syndactyly, Telecanthus, and Anogenital and Renal Malformations
CDT1	Chromatin licensing and DNA replication 1 (DNA replication - G1 phase)	Meier-Gorlin Syndrome 4 and Meier-Gorlin Syndrome
GAS8	Gene growth arrest specific 8 (tumor suppressor protein)	Ciliary Dyskinesia, Primary, 33 and Primary Ciliary Dyskinesia
TCF25	Transcription factor 25 (embryonic development)	Toe Syndactyly, Telecanthus, and Anogenital and Renal Malformations and Vulvovaginitis
ZNF276	Zinc Finger protein 276 (transcriptional regulation)	Fanconi Anemia, Complementation Group A
ZNF 778	Zinc Finger protein 778	Microdeletion Syndrome and Kbg Syndrome
ERG	ETS transcription factor	Regulators of embryonic development, cell proliferation, differentiation, angiogenesis, inflammation, and apoptosis

Table 4. WTK1 genes that were affected by ionizing radiation, their function/role, and pathology associated with aberrant gene expression.

Affected Gene	Function/role	Pathology
MOV10	RISC Complex RNA helicase (gene silencing)	Hepatitis and Autism Spectrum Disorder
PPM1J	Protein Phosphatase, Mg^{2+}/Mn^{2+} Dependent 1J (direct p53 effector protein)	Hepatocellular carcinoma
ST7L	Suppression Of Tumorigenicity 7 Like	Deleted and rearranged in a variety of cancers
CAMTA1	Calmodulin Binding Transcription Activator 1	Cerebellar Dysfunction with Variable Cognitive and Behavioral Abnormalities and Epithelioid Hemangioendothelioma
CISH	Cytokine Inducible SH2 Containing Protein (member of the SOC family of suppressors)	Bacteremia 2 and Malaria
MAPKAPK3	MAPK Activated Protein Kinase 3 (regulates cellular processes such as proliferation, differention, and cell-cycle progression)	Macular Dystrophy, Patterned, 3 and Bacteremia 2
PPP2R2C	Protein Phosphatase 2 Regulatory Subunit Bgamma (implicated in the negative control of cell growth and division)	Inflammatory Bowel Disease 3
GTF2H4	General Transcription Factor IIH Subunit 4 (involved in general and transcription-coupled repair)	Trichothiodystrophy and Cockayne Syndrome
CCDC6	Coiled-Coil Domain Containing 6 (possible tumor suppressor protein)	Differentiated Thyroid Carcinoma and Papillary Carcinoma
R3HCC1L	R3H Domain And Coiled-Coil Containing 1 (growth inhibition and differentiation)	Unknown
ENDOU	Endonuclease, Poly(U) Specific (Regulate B-cell activation-induced cell death)	Rapp-Hodgkin Syndrome and Noonan Syndrome 6
ZBTB21	Zinc Finger and BTB Domain Containing 21 (DNA binding transcription repressor)	Intellectual Developmental Disorder, Autosomal Dominant 7 and Noonan Syndrome 1

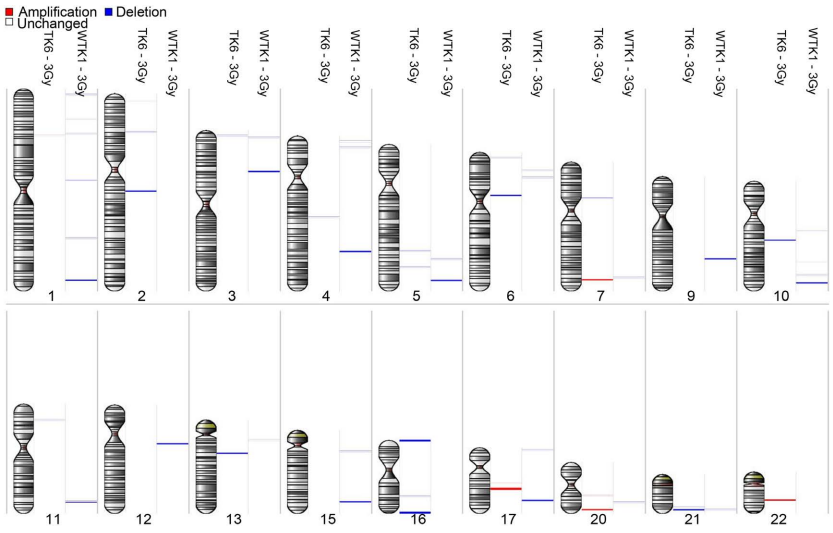

Figure 1. WTK1 genes that were affected by ionizing radiation, their function/role, and pathology associated with aberrant gene expression.

4. Discussion

In this study, we set out to understand the effects of ionizing radiation on two closely related cell lines: TK 6 (the wild type) and WTK 1 (the p53 mutant). Specifically, we wanted to investigate the copy-number variations between these two cell types to determine the level of impact that ionizing radiation has on genomic stability.

WTK1 contains the mutant p53 (M237I) at the thymidine kinase (tk) locus [37]. The p53 gene has been shown to be one of the most mutated genes as it relates to cancer. When comparing the level of mutability with the wild type p53 TK6 cells, it was shown that there is a 10-fold rate of hypermutability at the tk locus in the WTK1 cell line. p53 has also been shown to play a major role in maintaining genetic stability. p53 is a transcription factor that functions as a tumor suppressor, and p53 mutants have been shown to have loss of DNA binding function that prevents them from carrying out their regulatory role [38]. In addition, disruption of the interactions between the oncoprotein Mdm2, which promotes the rapid degradation of p53, with certain p53 mutants disrupts the p53 degradation pathway. This would indicate that mutant p53 is able to engage in aberrant interactions with other cellular factors. In fact, this has been shown to be the case and typically results in gain-of-function phenotypes [39] [40] [41].

The application of the Affymetrix mapping arrays on our cell lines has provided a wealth of information into the global impact on chromosomal instability after being subjected to ionizing radiation. Our results show a number of notable gene deletions and amplifications that are involved in general gene regulation, DNA damage repair, direct tumor suppression, regulation of the cell cycle and posttranslational modifications. The impact of abnormal gene expression, or, conversely, loss of expression, could potentially lead to a unique set of pathologies (see for example **Table 3** and **Table 4**).

Finally, we have demonstrated that WTK1 and TK6 do not share any genes that have been impacted in the same way by the irradiation. Instead, 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. The most notable differences are that TK6 is mostly affected in the areas of gene regulation and cell-cycle control, whereas WTK1 is mainly affected in tumor suppression and cell-cycle control. 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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