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Short Report

X Ray-Induced Insulinoma Cell Line Rin-5F Has a Novel Mutation Site, C.A1459G (P.T487A), in Death Domain Associated Protein (DAXX) Gene

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Abstract

A popular toxicological and pharmacological research cell line is the insulin-secreting pancreatic cell line Rin-5F. The cell line originates from insulinomas induced by X-ray exposure. The author of this report looked at the mutation status of the DAXX gene in the Rin-5F cell line clone. The complete DNA and RNA were extracted from the cultivated cells as well. Double-stranded cDNA was then synthesized using the RNA template. Sequencing was done using a 3730xl DNA Analyzer. In the present study, c.A1459G (p.T487A) in Exon 5 in the DAXX gene was detected in Rin-5F cell lines, one of the X-ray-induced insulinomas. An NCBI homology search reveals that the 487 amino acid site in rats is the 497 amino acid of humans, based on the genomic cDNA homology between rats and humans. In humans, the COSMIC database suggests that mutations involving 497 amino acids have not been detected in all human cancers. However, the mutation of 496 amino acids was detected in human stomach and colon cancers. This is the first account of the DAXX gene's state in a cell line created by exposure to X-rays. This may point to the need for additional data and research on unique gene alterations involved in the development of X-ray-induced insulinoma tumors.

Keywords

Daxx; insulinoma; Rin-5F

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1. Introduction

Despite being a relatively uncommon neuroendocrine malignancy, pancreatic neuroendocrine tumors make up 1-2% of pancreatic tumors and are currently the second most prevalent pancreatic ductal adenocarcinoma-related epithelial neoplasm [1]. The predominant mutations observed in pancreatic neuroendocrine tumors have been attributed to those associated with multiple endocrine neoplasia type 1 (MEN1), α-thalassemia/mental retardation syndrome X-linked protein (ATRX), and death domain associated protein (DAXX), constituting approximately 40%, 10%, and 20% of tumors, respectively [2]. These genes are involved in the remodeling of chromatin. A histone methyltransferase complex called MEN1 preferentially methylates histone H3's Lysine 4 and acts as a transcriptional regulator. Through a connection between ATRX and DAXX, histone H3.3-containing nucleosomes are deposited in the centromeric and telomeric regions of the genome [3].

The DAXX gene on rat chromosome 20 is related to the human 84.5% amino acid homologous DAXX gene (https://www.ncbi.nlm.nih.gov/gene/140926). The protein rat DAXX has 730 amino acids and has an estimated molecular weight of 80.71 kDa. Previously, the mutation in the DAXX gene within rat insulinoma cell lines has not been investigated. A popular toxicological and pharmacological research cell line is the insulin-secreting pancreatic cell line Rin-5F. The cell line originates from insulinomas induced by exposure to X-rays. The author of this report looked at the mutation status of the Rin-5F cell line clone and its genomic DNA and cDNA.

2. Materials and Methods

A continuous cell line identified as Rin-5F has been observed to retain numerous pancreatic beta-cell properties, notably the capacity for insulin secretion. These cells were grown using previously described techniques, and complete DNA and RNA were extracted from the cultivated cells. Double-stranded cDNA was subsequently synthesized utilizing the RNA template [4, 5]. National Center for Biotechnology Information (NCBI) Database has suggested that rat DAXX has 10 Exons (https://www.ncbi.nlm.nih.gov/gene/140926), but in fact, it has 7 Exons comparing sequence data of the genomic DNA; NCBI Reference Sequence: NC_051355.1, and the cDNA; NCBI Reference Sequence: NM_080891.2. Table 1 displays the forward and reverse primers. In order to facilitate sub-cloning into the pBlueScript II SK (+) vector (Stratagene, Santa Clara, California), XhoI and EcoRI sites were incorporated into the primer at the 5' and 3' ends, respectively, for Exon 1-7 and the cDNA of the DAXX gene. It used PrimeSTAR® HS DNA Polymerase from Takara Bio Inc. in Kusatsu, Japan, and KOD-Plus-Neo® from TOYOBO. Inc. in Osaka, Japan, the exons 1-7 and cDNA at DAXX were cloned. PCR was conducted as previously described [4, 5]. At Eurofins Genomics in Tokyo, Japan, sequencing was done using a 3730xl DNA Analyzer (Thermo Fisher Scientific, Tokyo, Japan).

Table 1 Sequence of the Forward and Reverse Primers Used for the Amplification and Sequencing of Rat DAXX Gene.

Primer Sets		Sequence
Exon 1	Forward	GATGAATTCGGAGGGGCTTTTCCTGAGTC
	Reverse	ATACTCGAGCCTTGGGATCTGCCCAATCA
Exon 2	Forward	ATAGGATCCGGCTTCCTCACTGATTCCCT
	Reverse	GATGTCGACGGAAACAGGGTCAGAGCAGT
Exon 3	Forward	GCCGAATTCTTTCAGGTGGAACAGAGGCA
	Reverse	ATACTCGAGCTGGGGGAAAGAGGAGTCCC
Exon 4	Forward	GACGAATTCCGGATTCCGGTGAGGTATGG
	Reverse	ATACTCGAGAGCTCAGAAAGCTGGGACAC
Exon 5	Forward	GACGAATTCTCTGTCCGACATGACCTCCT
	Reverse	ATACTCGAGGGACACCAGGTTCCCTTCAG
Exon 6	Forward	GACGAATTCGTTCCCTCCCCACCATCTTG
	Reverse	ATACTCGAGAGTGATCAGTTGGGAGCAGC
Exon 7	Forward	GGGCGAATTCAGGCCGTGTAAAACAAAAAGTG
	Reverse	ATACTCGAGCAAAACGGGAGGAGCTCTGT
cDNA	Forward	ATACTCGAGATGGCCACCGATGACAGCATCATT
	Reverse	GGCGAATTCCTAATCAGAGTCTGAGAGCACGAT

All experimental procedures followed the University's Guidelines for Recombinant DNA Experiments, of which the author is a member. The University's Safety Committee approved the Protocols for Recombinant DNA Experiments (approval number 1714).

3. Results

PrimeSTAR® HS DNA Polymerase and KOD-Plus-Neo® were two distinct PCR enzymes used in this investigation. These techniques produced identical results for the genomic DNA and cDNA in the DAXX gene in Rin-5F cell lines. These mutations were c.C33T (silent mutation) and c.C96T (silent mutation) in Exon 1, c.T381C (silent mutation) in Exon 2, and c.A1459G (p.T487A) and c.G1521A (silent mutation) in Exon 5 (Figure 1A).

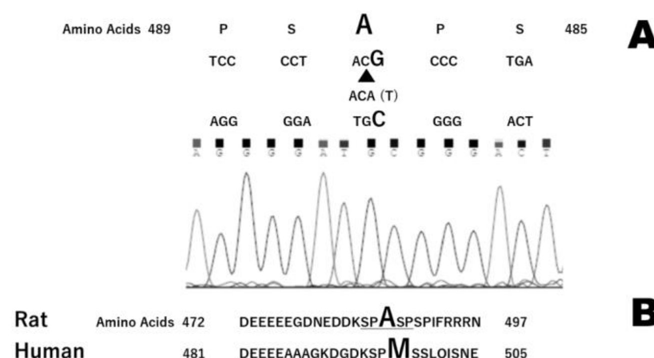

Figure 1 A. c. A1459G (p.T487A) in Exon 5 in the DAXX gene was detected in Rin-5F cell lines. **B.** The NCBI homology study suggested that 487 rat amino acid positions (A: Alanine) correspond to 497 human amino acids (M: Methionine).

4. Discussions

It has been noted that X-irradiation causes insulinomas in Sprague-Dawley (SD) rats [6, 7]. Because this X-irradiated insulinoma cell line (i.e., Rin-5F) has an amino acids mutation of the DAXX gene, the deposition of histone H3.3-containing nucleosomes in the centromeric and telomeric regions of the genome may have a role in the mechanism of X-irradiated insulinoma carcinogenesis.

Extensive validation is needed when reporting a single nucleotide polymorphism (SNP) as the sole mutation in a cell line. Validating such findings typically involves implementing a comprehensive workflow that ensures accurate results and minimizes the risk of false positives or artifacts. The SNP is typically detected through sequencing methods and compared to a reference genome as part of the initial identification process. This study used the NCBI Reference Sequence (NM_080891.2) as the reference genome. Next, as the confirmation, the SNP finding is confirmed using alternative methods to verify its presence in the cell line. The study used two PCR enzymes: PrimeSTAR® HS DNA Polymerase and KOD-Plus-Neo®. These PCR enzymes obtained the same results. Next, as in replication, the repeat SNP detection process on multiple samples of the same cell line is used to confirm the consistency of the mutation across replicates. The study used the low passage number of cells to prevent the effect of the replications. Next, as control experiments, the appropriate controls are needed to rule out sequencing errors or experimental artifacts. This study used the NCBI Reference Sequence (NM_080891.2) as the control genome. At last, as functional validation, the functional impact of the SNP is needed to assess the cell line's phenotype through other relevant experimental approaches. In the present study, four silent mutations belonging to Exons 1, 2, and 5 were detected. One missense mutation site belonged to Exon 5. Several studies have reported mutations within exon 5 of the DAXX gene in human pancreatic neuroendocrine tumors [8, 9]. The mutation of amino acids within this region has been correlated with the loss of the corresponding protein expression in the nucleus [9]. Therefore, there is a possibility that the mutation may be involved in the development of X-ray-induced insulinoma tumors.

An NCBI homology search (https://www.ncbi.nlm.nih.gov/gene/140926) reveals that the 487 amino acid site in the rat is the 497 amino acid of the human, based on the genomic cDNA homology between rat and human (Figure 1B). In humans, the COSMIC (the Catalogue Of Somatic Mutations In Cancer) (https://cancer.sanger.ac.uk/cosmic) suggested that the mutation of 497 amino acids was not detected in all human cancers. However, the mutation of 496 amino acids was detected in human stomach and colon cancers. Hence, there exists a possibility that this amino acid mutation may be associated with the effects of radiation, given its absence in human pancreatic neuroendocrine tumors.

Recent reports indicate that mutations in the DAXX gene impact telomere length, as demonstrated by a comprehensive genome analysis of various tumors. The gene encodes proteins that normally repress a telomerase-independent mechanism of telomere maintenance. The analysis found that tumors that expressed truncated versions of DAXX had more telomere DNA than tumors expressing the full-length proteins, suggesting that truncating mutations in DAXX may promote telomere maintenance in cancer [10]. Consistent with this, a phase I study has been reported that patients with tumors harboring the mutations in the DAXX gene receive Tuvusertib (Merck KGaA, Darmstadt, Germany), a telomere maintenance inhibitor, in patients with solid tumors [11].

5. Conclusion

This represents the initial documentation of the status of the DAXX gene within a cell line induced by X-ray exposure. These findings may underscore the necessity for further data and research concerning distinctive gene alterations implicated in the pathogenesis of X-ray-induced insulinoma tumors.

Abbreviations

ATRX	α-thalassemia/mental retardation syndrome X-linked protein
DAXX	death domain associated protein
NCBI	National Center for Biotechnology Information
MEN1	Multiple endocrine neoplasia type 1

Author Contributions

TK devised the study, gathered data, carried out data, analyzed findings, and authored and revised the initial and final manuscripts. The final manuscript was read and approved by the author.

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Competing Interests

The author declares no conflict of interest.

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