Investigation of Unknown Causes of Uveal Melanoma Uncovers Seven Recurrent Genetic Mutations

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Abstract

The purpose of this research was to investigate which genetic mutations are responsible for Uveal Melanoma (UM), a rare subtype of melanoma but the most frequent primary cancer of the eye. Genome data of UM patients was obtained from U.S. National Institute of Health's (NIH) National Library of Medicine. Data was obtained from samples that were surgically collected from eye enucleations or resected from liver metastases. The DNA sequence from the cancerous cells was compared to a reference DNA sequence (from normal tissue pairs) to identify any nucleotide base pair mismatches. Gene functions of mutated genes were studied to investigate possible causal links to cancer, such as anomalies in genes that coded for proteins with a known role in tumor repression. A total of 130 genetic mutations were discovered (seven recurrent and 123 non-recurrent), with most mutations occurring in chromosomes 3 and 23. Recurrent mutations varied from 8.7% to 17.39% occurrence in the UM patient sample. The recurring genetic mutations were observed as missense mutations in the following genes: ALG1L2, DMD, IL1RAPL2, KIAA0825, LOC440040, NXF2, and PHYHD1. The research revealed UM is a heterogenous disease with homozygous mutations and is a recessive disorder.

Background

Melanoma is a life-threatening malignancy that affects melanocytes (pigment-producing cells) found throughout the body. Melanomas are of two types: cutaneous and non-cutaneous. Cutaneous melanomas, which account for approximately 95% of all melanomas, originate in the pigment-producing cells of the skin. On the other hand, non-cutaneous (non-skin) melanomas affect other regions of the body including the eyes and mucous membranes, such as those present in nasal passages and the oral cavity.

Although it is a rare disease, uveal melanoma is the most common form of non-cutaneous melanoma, and it is the most frequent primary cancer of the eye in the adult [1]. Uveal melanoma is known to affect ˜7000 individuals worldwide annually [2], with incidence rates ranging from 0.2 to 0.3 per million individuals in African and Asian populations to up to 6 per million in white populations [3]. Diagnosis usually occurs at age 60, and this cancer is more prevalent among Caucasians.

In most cases, UM forms in the choroid: the vascular layer of the eye lying between the sclera and the retina. Symptoms that may be exhibited by UM patients include but are not limited to: variable and painless visual disturbances, discoloration of the iris, change the shape of the pupil, or loss of peripheral vision.

Risk of Metastasis

One aspect that contributes to the deadliness of melanomas is the risk of metastasis. Metastasis is the migration of cancerous cells through the bloodstream, which leads to the development of tumors elsewhere in the body. These tumors can cause tissue damage and other widespread effects which accelerate the death of the cancer patient. In the case uveal melanoma, the liver is the most common organ affected by metastasis, which occurs in 80% of cases [4]. Although regular metastasis can be detected, undetectable

micrometastases may occur, in which a small collection of cancerous cells spread to other parts of the body via the lymphovascular system. Micrometastasis is a significant risk to all UM patients, which is why the patient should receive immediate treatment after diagnosis.

Uveal Melanoma Causes and Treatments

Unfortunately, the causes for uveal melanoma are currently unclear. However, some studies have revealed that the DNA present within the cancerous cells showed alterations on chromosomes 1,3,6, and 8. They have also found that genes BAP1, SF3B1, GNAQ, and GNA11 seem to play a role in the development of uveal melanoma [5,6]. Although these studies did uncover some genetic errors that may be responsible, they had only analyzed chromosomal rearrangements, and not specific coding mutations. Currently there are no effective therapies for the treatment of UM patients.

Introduction

This project aimed to determine what genetic mutations (resulting from coding errors) could be responsible for the development of uveal melanoma. Within this study, genetic alterations resulting from chromosomal rearrangements were disregarded, but base pair mismatches causing missense, nonsense, or frameshift mutations were considered. Some patients had also experienced metastasis of cancerous cells through the bloodstream; data from their sample genomes were considered separately in an effort to identify any genetic mutations accelerating metastasis. The basic procedures for this study involve the comparison of a mutated DNA sequence (found in cancerous cells) with the matched normal sequence (found in healthy, unaffected cells).

Patient Genome Data

All data for this experiment was collected from Complete Genomics Inc. (publicly available data published by US National Center for Biotechnology Information (NCBI) [5]. Sample genome data was obtained from 32 UM patients, and the data package included 32 data files (23 primary PUM files, 9 metastases MUM files). The UM patient data was in excel format, and each file included a sample genome DNA sequence (matched normal) and a mutated allele 2 sequence from each patient.

Methodology

For the analysis, the SNV (Single-Nucleotide Variant) files were examined from the data package. The data files contained information which provided the locus of each mutation, the zygosity, variation type, and the gene on which it occurred. The 'Match' column was not present within the data and was created with a formula as a detection method for any coding mutations. The formula was an IF function which compared each cell from the reference sequence with the corresponding cell from the allele 2 sequence. Whenever there was a mismatch, the function would automatically flag each mutation with an 'X' symbol in the corresponding cell within the 'Match' column. After the IF function was imported into the 'Match' column, it was applied to the entire genome to search for any mutations. Once the comparison was complete, the 'Match' column was filtered out to only show the base pair mismatches (occurred only when an 'X' was present in the 'Match' column) [Figure 1]. These procedures were followed for both the primary (PUM) and metastases (MUM) files.

>locus	ploidy	chromosom	begin	end	zygosit	varTyp	Match	referer	allele2Sequenc	gene_nam
2360495	2	chr3	1.9E+07	1.9E+07	hom	snp	X	G	A	NA
2362751	2	chr3	1.9E+07	1.9E+07	hom	snp	X	C	T	NA
2409953	2	chr3	2.9E+07	2.9E+07	half	sub	X	ATAACAG	?	NA
2424046	2	chr3	3.2E+07	3.2E+07	half	snp	X	T	?	CMTM8;
2444320	2	chr3	3.6E+07	3.6E+07	half	snp	X	T	?	STAC;
2468681	2	chr3	4.2E+07	4.2E+07	hom	snp	X	A	G	NA
2509341	2	chr3	5.2E+07	5.2E+07	half	sub	X	CTGCGGT	?T	BAP1;PHF7;
2521149	2	chr3	5.5E+07	5.5E+07	hom	snp	X	G	A	NA
2550459	2	chr3	6.1E+07	6.1E+07	half	snp	X	CCC	?	NA
2550469	2	chr3	6.1E+07	6.1E+07	half	snp	X	G	?	NA
2550471	2	chr3	6.1E+07	6.1E+07	half	snp	X	C	?	NA
2550473	2	chr3	6.1E+07	6.1E+07	half	snp	X	C	?	NA
2550475	2	chr3	6.1E+07	6.1E+07	half	snp	X	G	?	NA
2550477	2	chr3	6.1E+07	6.1E+07	half	snp	X	G	?	NA
2550479	2	chr3	6.1E+07	6.1E+07	half	snp	X	T	?	NA
2550493	2	chr3	6.1E+07	6.1E+07	half	snp	X	AGC	?	NA
2550495	2	chr3	6.1E+07	6.1E+07	half	snp	X	G	?	NA
2550497	2	chr3	6.1E+07	6.1E+07	half	snp	X	A	?	NA
2581487	2	chr3	6.9E+07	6.9E+07	hom	snp	X	C	T	FAM19A4;
2607967	2	chr3	7.5E+07	7.5E+07	half	snp	X	A	?	NA
2658020	2	chr3	8.1E+07	8.1E+07	hom	snp	X	G	C	NA
2669943	2	chr3	8.4E+07	8.4E+07	half	del	X	CACTACA	?ACACATGCTTATA	NA
2850500	2	chr3	1.3E+08	1.3E+08	hom	snp	X	G	C	ZNF148;
2875078	2	chr3	1.3E+08	1.3E+08	half	snp	X	A	?	NA
2875680	2	chr3	1.3E+08	1.3E+08	half	snp	X	ACCTA	?	NA
2876044	2	chr3	1.3E+08	1.3E+08	half	snp	X	C	?	NA
2876076	2	chr3	1.3E+08	1.3E+08	half	snp	X	CAA	?	NA
2876356	2	chr3	1.3E+08	1.3E+08	hom	snp	X	T	C	ALG1L2;
2876384	2	chr3	1.3E+08	1.3E+08	half	snp	X	CG	?	ALG1L2;

All of the mutations that occurred were noted along with their respective chromosome number, zygosity, and gene name. The gender of each patient was also taken into consideration in order to determine whether uveal melanoma is a sex-linked disorder. However, the variation type was not significant, as that had already been investigated by other studies.

Pareto analysis was performed to visualize the frequency of the specific genetic mutations, as well as the chromosomes on which they were present. A Pareto chart is a bar chart in which the bars are ordered from highest frequency of occurrence to lowest frequency of occurrence [7]. This statistical technique was helpful in understanding the results of the study. An individual pareto chart regarding chromosome involvement was generated for both the primary and metastases samples each [Figure 2 & 3]. These charts provided information on the number and frequency of genetic mutations per chromosome in decreasing order. Pareto analysis of mutated genes was only run for the primary samples [Figure 4] because the results revealed that there were no recurring genetic mutations among the patients whose cells had undergone metastasis.

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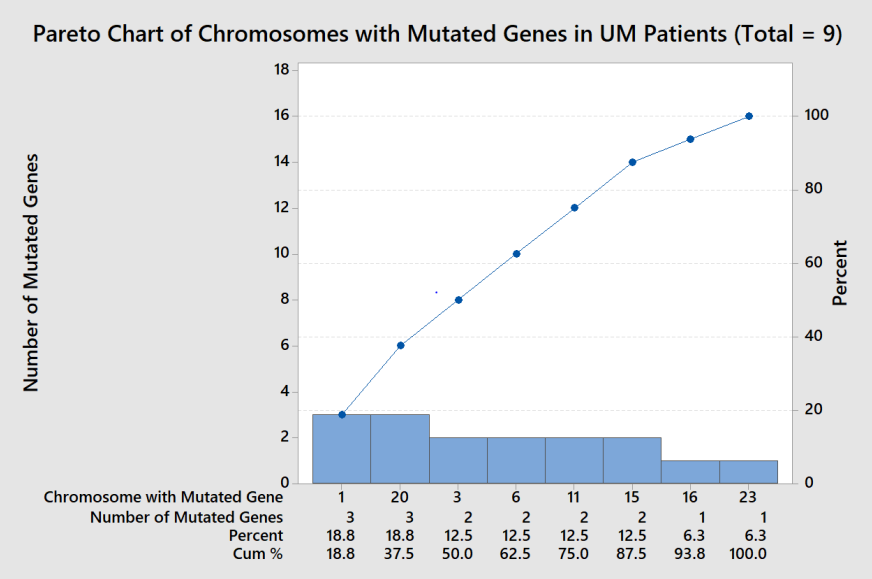
(Figure 2) Pareto chart that depicts the frequency of chromosomes with mutated genes in uveal melanoma patients (primary samples).



(Figure 3) Pareto analysis for the frequency of chromosomes with mutated genes in uveal melanoma patients (metastases samples).

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Results

Following the analysis, 130 unique genetic mutations were observed in all the UM patients. Out of those 130 mutations, 7 were recurrent in the primary samples, and none were recurrent in the metastases samples. The 7 most recurrent mutations were ALG1L2, DMD, IL1RAPL2, KIA0825, LOC440040, NXF2, and PHYHD1. ALG1L2, being the most frequent genetic mutation, was observed in 4 patients out of the 23 primary samples (32 samples total). This indicates a 17.39% recurrence, which is quite high considering that there are hundreds of thousands of genes within the human genome. It was also observed that most of the genetic mutations were present on chromosomes 3, 5, 9, 11, and 23 (X chromosome), which reconfirms previous findings of chromosomes 1 and 3 being involved, and identifies chromosomes 5, 9, and 23 as new factors. The pareto analyses revealed that the X chromosome and chromosome 3 experienced the most genetic mutations, totaling at around 75 mutations combined (primary samples) [Figure 2].

Conclusions

From this study, it can be concluded that uveal melanoma is a heterogenous disease because the genetic mutations were predominantly non-recurrent (130 total). This aggressive and heterogenous nature is a very common characteristic of most types of melanomas and serves as important information for determining what would be an appropriate form of treatment for UM patients. The pareto analysis uncovered that the frequency of the recurrent genetic mutations ranged from 8.7% to 17.39%, indicating that these mutations were likely caused by the cancer and not due to single nucleotide polymorphism. To confirm this inference, the functions of all the mutated genes were researched, and the recurrent ones were studied extensively. For example, the most recurrent gene, ALG1L2, codes for transferase activity, or transfer of glycosyl groups. This activity plays a critical role in determining the structure, stability, and function of a protein. It was also found that most of the non-recurrent genes that were mutated coded for transcription factors and tumor suppressors – proteins that, when defective, are known to cause other cancers.

The genetic mutations were homozygous, meaning that uveal melanoma is a recessive disorder, which explains the rarity of this cancer. They were observed to be missense mutations, resulting from single base mismatches within the protein coding regions of the DNA. The other two type, frameshift and nonsense mutations, were not found within any of the samples that were analyzed. All of the recurrent genetic mutation that were found in this study have not been listed in any source of literature, suggesting that these are potentially new and yet undiscovered mutations responsible for uveal melanoma, and likely responsible for other cancers (as the same genetic mutations are often responsible for multiple types of cancers, such as genetic mutation in BRAF V600E, TP53, and CDKN2A have high recurrence in cutaneous melanomas).

Significance of Research & Future Work

One major implication of this study is that it suggests the best treatment approach for people suffering from UM. Since the results show that UM is caused by several *unique* genetic mutations, thus implying its heterogenous nature, a targeted therapy would not be the best approach. Targeted therapies are developed to target and inhibit the function of a specific gene or defective protein that results from genetic mutations. For example, Vitrakvi ® (larotrectinib) from Bayer is a medication for solid tumors that inhibits TRK (tyrosine kinase), a protein which promotes cancer. TRK is produced as a result of the fusion of two genes due to an underlying genetic mutation in NTRK (neurotrophic receptor tyrosine kinase). The best treatment option for UM would be some form of immunotherapy, which prevents disease by stimulating and enhancing the immune system to fight against dysfunctional proteins caused by mutations. Immunotherapy would be effective in reducing the cumulative effects of all these harmful genes and proteins. However, targeted therapy could be used to inactivate specific genes which exhibit mutations frequently in UM.

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DATA SOURCES

- *Reference:***https://www.ncbi.nlm.nih.gov/pubmed/27745836**
- *Data files:***https://drive.switch.ch/index.php/s/7GjD2zQEoPOyaj9**