DISTRIBUTION OF MOBILE GENETIC ELEMENTS IN CANCER GENES

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ABSTRACT

Mobile genetic elements (MGEs) represent a large portion of the human genome. Its ability to change their position within the genome has contributed to evolution, however, the same has also resulted in several mutations. Many of such mutations are known to cause exon skipping or premature truncation that result in non-functional or dysfunctional protein, leading to cancer. Here, in this study we aim to determine the distribution of MGEs in cancer-associated genes as compared to non-cancer associated genes. We curated a list of genes for both the categories and downloaded the nucleotide sequences of these genes and ran on RepeatMasker to identify the MGEs in each gene. All the data generated with respect to each gene was parsed and compared. The results showed that the number and the sequence length covered by the identified MGEs in cancer-associated genes were comparatively high. The abundance of MGEs may be correlated with the high risk of deletion/insertion of large DNA segments in these genes, that results in higher risk of cancer. Further studies need to be performed for better clarity on these associations.

Keywords: Mobile genetic elements, RepeatMasker, Oncogene, Proto-oncogene, Cancer

INTRODUCTION

MGEs are the DNA segments that can change their location within the genome. They are present in the genomes of most of organisms which includes, prokaryotes as well as eukaryotes. In humans, they occupy large fraction (>50 %) of the genome [Cao et al. 2020]. MGEs can be broadly classified into retrotransposons class (Class I) and DNA transposons (Class II), based on their mode of transmission. Out of two classes, retrotransposon variety is the most widely spread MGEs in mammals. Retrotransposons can be further categorized into Long Terminal Repeats (LTRs) and non-LTRs [Anwar et al. 2017]. Autonomous non-long terminal repeat retrotransposons are commonly referred to as long interspersed elements (LINEs). Short non-autonomous elements that borrow the LINE machinery are called SINES [Bakre et al 2005].

Though, majority of the MGEs are inactive in human genome, there are varieties of non-LTRs (like LINEs and SINEs) that are capable of generating mutations. These active MGEs has potential to disrupt genes and play role in chromosomal rearrangements [Anwar et al. 2017]. MGE-generated mutations have shown to be associated with diseases affecting humans such as Mankes syndrome, X-linked agammaglobulinemia, Dent disease, hemophilia, cholinesterase deficiency, hereditary nonpolyposis colorectal cancer, familial adenomatous polyposis, leukemia, and cystic fibrosis [Hancks et al. 2012]. In a study performed by Wimmer et al. (2011), on neurofibromatosis type 1 patients, 18 unrelated patients were identified with MGE insertions in *NF1* gene in exonic or intronic region. Studies have also shown that the insertion of mobile DNA in genes might promote exon skipping, resulting in either reduced or no expression of the affected gene(s) [Payer et al. 2019; Nikopoulos et al. 2019; Jourdy et al. 2018]. In 1988, Kazazian et al. discovered that TE insertion was responsible for causation of hemophilia A. Large-scale genomic projects, such as the ENCyclopedia Of DNA Elements (ENCODE) and Functional Annotation of Mouse (FANTOM) projects as well as independent studies have indicated that TEs are active in a highly cell type-specific manner and control their own cell-specific transcription as well as the transcription of neighboring genes [Faulkner et al. 2009; Djebali et al. 2012; Thurman et al. 2012]. Previously, our group identified unique patterns/signals at the insertion sites flanking MGEs using machine learning [Rawal and Ramaswamy 2011]. In this work, we found that signals present in exonic regions are different from the intronic regions of the genes. All these studies indicate the importance of understanding the role of MGEs insertions in human genes. Here, in this study we aim to elucidate the distribution of MGEs on oncogenes (proto-oncogene) and also to compare it with the non-cancer genes.

MATERIAL AND METHOD

Datasets:

We extracted a list of proto-oncogene or oncogenes from open source databases, including OMIM (<https://omim.org/>), Genetics-MedlinePlus (<https://medlineplus.gov/genetics/gene/>), and Cancer Genetics Web (<http://www.cancerindex.org/geneweb/>) [labelled as Set T]. We also downloaded a whole list of human genes from NCBI database ([https://www.ncbi.nlm.nih.gov/gene/?term=human\[organism\]](https://www.ncbi.nlm.nih.gov/gene/?term=human[organism])) [Set W]. Next, we subtracted Set W by removing the Set T genes, as well as removed genes without RefSeq ID. The filtered list contains non cancer genes [Set N] as control dataset.

Nucleotide Sequence Download

We extracted the nucleotide sequences of genes in Set T and N using Edirect utilities. We divided Set N into 5 different sub-sets, N1, N2, N3, N4 and N5. Each subset consist of randomly selected 1082 genes as control dataset.

MGE Detection

We used RepeatMasker to identify and characterize the MGEs on gene sequences belonging to different set. Next, we used in-house scripts (Bash) that parsed RepeatMasker output files.

Statistical Analysis

We computed standard statistical measures such as mean and standard deviations for the data obtained from Repeatmasker. Next, we compared these values in different sets (namely T, W and N) using T-test (P values <0.05).

RESULTS

Data Collection:

We identified 1164 genes in Set T that are reported to be associated with cancer or tumor (See Supplementary Table 1). We also downloaded a list of 61746 human genes in Set W (See Supplementary Table 1). We filtered Set W for all the matches with tumor/cancer keywords. We also filtered genes having no information on RefSeq ID leading to a total of 9994 genes in Set N (See Supplementary Table 1).

Nucleotide sequence download

From Set T, we could extract the nucleotide sequences of 1082 genes out of 1164. This was because of the unavailability of the nucleotide sequence for few genes or the alias of gene name might be used in Edirect to refer to the gene (See File 4). Similarly, for Set N, we could download 9454 sequences out of 9994 genes present in the list.

MGE Detection

All the extracted nucleotide sequences were run on RepeatMasker to identify the MGE as well as other details such as coordinates, type, score etc. (see Table 1). We found that the number of MGEs in Set T (cancer genes) were higher as compared to Set N (control) (see Figure 1). We also compared the percentage of sequence covered by MGEs and the sequence length covered by MGEs in both the sets (see Figure 2 and Figure 3).

Since the number of genes in Set N (control dataset) were more than that of genes of Set T, we constructed 5 subsets of Set N, each with 1082 genes. During the analysis, we found that the frequency of MGEs in Set T was higher as compared to all the other subsets of Set N (see Table 2).

CONCLUSION AND FUTURE PERSPECTIVE

In this study, we performed a comparison between the distribution of MGEs in genes that are associated with cancer and genes that are not associated with cancer. The results have shown the abundance of MGEs in cancer-associated genes. Though the reasons for this abundance is not known but it can be hypothesized that the presence of these MGEs can make these genes amenable for large deletions or duplications/insertions that could lead to pathogenic mutations in the gene, resulting in cancer. Further studies are needed to explore the role of MGEs in genes expression and cancer genomics.

Contribution of Authors

This study was conducted in the overall guidance of KR, who contributed in protocol, critical evaluation of data and manuscript. Data was generated by TKB. Data analysis, cross-checking and manuscript writing was done by PP. All the statistical analysis was done by PP, TKM and KR. All the authors are responsible for the content of the manuscript.

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REFERENCES

1. Cao X, Zhang Y, Payer LM, et al. Polymorphic mobile element insertions contribute to gene expression and alternative splicing in human tissues. *Genome Biol.* 2020;21(1):185. Published 2020 Jul 27. doi:10.1186/s13059-020-02101-4.
2. Anwar SL, Wulaningsih W, Lehmann U. Transposable Elements in Human Cancer: Causes and Consequences of Dereglulation. *Int J Mol Sci.* 2017;18(5):974. Published 2017 May 4. doi:10.3390/ijms18050974
3. Hancks DC, Kazazian HH Jr. Active human retrotransposons: variation and disease. *Curr Opin Genet Dev.* 2012;22(3):191-203. doi:10.1016/j.gde.2012.02.006
4. Wimmer K, Callens T, Wernstedt A, Messiaen L. The NF1 gene contains hotspots for L1 endonuclease-dependent de novo insertion. *PLoS Genet.* 2011;7(11):e1002371. doi:10.1371/journal.pgen.1002371
5. Payer LM, Steranka JP, Ardeljan D, et al. Alu insertion variants alter mRNA splicing. *Nucleic Acids Res.* 2019;47(1):421-431. doi:10.1093/nar/gky1086
6. Nikopoulos K, Cisarova K, Quinodoz M, et al. A frequent variant in the Japanese population determines quasi-Mendelian inheritance of rare retinal ciliopathy. *Nat Commun.* 2019;10(1):2884. Published 2019 Jun 28. doi:10.1038/s41467-019-10746-4
7. Jourdy Y, Janin A, Fretigny M, et al. Recurrent F8 Intronic Deletion Found in Mild Hemophilia A Causes Alu Exonization. *Am J Hum Genet.* 2018;102(2):199-206. doi:10.1016/j.ajhg.2017.12.010

8. Faulkner GJ, Kimura Y, Daub CO, Wani S, Plessy C, Irvine KM, Schroder K, Cloonan N, Steptoe AL, Lassmann T, Waki K, Hornig N, Arakawa T, Takahashi H, Kawai J, Forrest AR, Suzuki H, Hayashizaki Y, Hume DA, Orlando V, Grimmond SM, Carninci P. The regulated retrotransposon transcriptome of mammalian cells. *Nat Genet.* 2009 May;41(5):563-71. doi: 10.1038/ng.368. Epub 2009 Apr 19. PMID: 19377475.
9. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, Tanzer A, Lagarde J, Lin W, Schlesinger F, Xue C, Marinov GK, Khatun J, Williams BA, Zaleski C, Rozowsky J, Röder M, Kokocinski F, Abdelhamid RF, Alioto T, Antoshechkin I, Baer MT, Bar NS, Batut P, Bell K, Bell I, Chakraborty S, Chen X, Chrast J, Curado J, Derrien T, Drenkow J, Dumais E, Dumais J, Duttagupta R, Falconnet E, Fastuca M, Fejes-Toth K, Ferreira P, Foissac S, Fullwood MJ, Gao H, Gonzalez D, Gordon A, Gunawardena H, Howald C, Jha S, Johnson R, Kapranov P, King B, Kingswood C, Luo OJ, Park E, Persaud K, Preall JB, Ribeca P, Risk B, Robyr D, Sammeth M, Schaffer L, See LH, Shahab A, Skancke J, Suzuki AM, Takahashi H, Tilgner H, Trout D, Walters N, Wang H, Wrobel J, Yu Y, Ruan X, Hayashizaki Y, Harrow J, Gerstein M, Hubbard T, Reymond A, Antonarakis SE, Hannon G, Giddings MC, Ruan Y, Wold B, Carninci P, Guigó R, Gingeras TR. Landscape of transcription in human cells. *Nature.* 2012 Sep 6;489(7414):101-8. doi: 10.1038/nature11233. PMID: 22955620; PMCID: PMC3684276.
10. Thurman RE, Rynes E, Humbert R, Vierstra J, Maurano MT, Haugen E, Sheffield NC, Stergachis AB, Wang H, Vernot B, Garg K, John S, Sandstrom R, Bates D, Boatman L, Canfield TK, Diegel M, Dunn D, Ebersol AK, Frum T, Giste E, Johnson AK, Johnson EM, Kutysavin T, Lajoie B, Lee BK, Lee K, London D, Lotakis D, Neph S, Neri F, Nguyen ED, Qu H, Reynolds AP, Roach V, Safi A, Sanchez ME, Sanyal A, Shafer A, Simon JM, Song L, Vong S, Weaver M, Yan Y, Zhang Z, Zhang Z, Lenhard B, Tewari M, Dorschner MO, Hansen RS, Navas PA, Stamatoyannopoulos G, Iyer VR, Lieb JD, Sunyaev SR, Akey JM, Sabo PJ, Kaul R, Furey TS, Dekker J, Crawford GE, Stamatoyannopoulos JA. The accessible chromatin landscape of the human genome. *Nature.* 2012 Sep 6;489(7414):75-82. doi: 10.1038/nature11232. PMID: 22955617; PMCID: PMC3721348.

Table 1: Comparison of RepeatMasker results between Set T and Set N

PARAMETERS/GENE SETS	Set T	Set N
No. of genes	1082	9454
GC level (%)	45.80	46.43
Bases masked (bp)	42464.58	33748.54
Bases masked (%)	35.63	37.29
Number of SINES	66.37	47.33
Length of SINES (bp)	15896.55	11190.46
Percentage of SINES	16.99	16.73
Number of ALUs	45.45	31.08
Length of ALUs (bp)	12737.09	8717.72
Percentage of ALUs	14.15	13.57
Number of MIRs	20.52	16.01
Length of MIRs (bp)	3107.57	2441.83
Percentage of MIRs	2.80	3.14
Number of LINES	32.02	25.46
Length of LINES (bp)	15628.45	13881.68
Percentage of LINES	10.52	11.66
Number of LINE1	16.19	12.90
Length of LINE1 (bp)	11505.75	10429.40
Percentage of LINE1	7.10	7.96
Number of LINE2	13.31	10.62
Length of LINE2 (bp)	3550.82	2992.54
Percentage of LINE2	2.99	3.29
Number of L3/CR1	1.92	1.46
Length of L3/CR1 (bp)	429.94	334.75
Percentage of L3/CR1	0.32	0.32
Number of LTR	10.15	8.93
Length of LTR (bp)	4749.27	4270.76
Percentage of LTR	2.75	3.67
Number of ERVL	2.09	1.85
Length of ERVL (bp)	990.63	882.81
Percentage of ERVL	0.59	0.75
Number of ERVL-MaLRs	5.46	4.74
Length of ERVL-MaLRs (bp)	2241.70	1969.89
Percentage of ERVL-MaLRs	1.27	1.49
Number of ERV_classI	1.93	1.73
Length of ERV_classI (bp)	1243.02	1172.28
Percentage of ERV_classI	0.71	1.15

Number of ERV_classII	0.14	0.12
Length of ERV_classII (bp)	130.39	118.75
Percentage of ERV_classII	0.10	0.16
Number of DNA_elements	17.14	12.52
Length of DNA_elements (bp)	4014.95	2938.19
Percentage of DNA_elements	3.05	2.97
Number of hAT-Charlie (No.)	9.22	6.68
Length of hAT-Charlie (bp)	1885.93	1349.90
Percentage of hAT-Charlie	1.48	1.45
Number of TcMar-Tigger	4.33	3.08
Length of TcMar-Tigger (bp)	1455.16	1063.87
Percentage of TcMar-Tigger	1.13	0.98
Number of Unclassified	2.78	1.85
Length of Unclassified (bp)	359.91	245.65
Percentage of Unclassified	0.36	0.36
Number of Total_interspersed_repeats	NA	NA
Length of Total_interspersed_repeats (bp)	40649.13	32526.74
Percentage of Total_interspersed_repeats	33.67	35.39
Number of Small_RNA	3.11	2.06
Length of Small_RNA	202.00	133.31
Percentage of Small_RNA	0.22	0.26
Number of Satellites	0.04	0.02
Length of Satellites (bp)	48.43	14.14
Percentage of Satellites	0.02	0.08
Number of Simple_repeats	27.75	19.11
Length of Simple_repeats (bp)	1389.35	947.15
Percentage of Simple_repeats	1.47	1.32
Number of Low_complexity	3.06	2.20
Length of Low_complexity (bp)	169.37	121.04
Percentage of Low_complexity	0.23	0.23

Table 2: Comparison of number of MGEs identified in Set T and different subsets of Set N

PARAMETERS/GENE SETS	Set T	Set N1	Set N2	Set N3	Set N4	Set N5
SINEs	66.37	48.54	45.95	46.93	47.89	48.02
ALUs	45.45	31.22	29.85	31.41	31.07	31.29
MIRs	20.52	17.07	15.87	15.32	16.58	16.48
LINEs	32.02	26.22	25.21	24.53	26.37	25.95
LINE1	16.19	13.13	12.74	12.49	13.24	13.07
LINE2	13.31	11.11	10.61	10.19	11.11	10.88
L3/CR1	1.92	1.52	1.39	1.39	1.51	1.53
LTR	10.15	9.06	9.27	8.40	9.28	9.04
ERVL	2.09	1.87	1.96	1.79	1.94	1.92
ERVL-MaLRs	5.46	4.82	4.92	4.40	4.87	4.78
ERV_classI	1.93	1.78	1.79	1.70	1.79	1.75
ERV_classII	0.14	0.12	0.12	0.11	0.14	0.12
DNA_elements	17.14	12.94	12.26	11.75	12.69	13.09
hAT-Charlie	9.22	6.86	6.53	6.22	6.66	7.04
TcMar-Tigger	4.33	3.26	3.08	3.01	3.20	3.14
Unclassified	2.78	1.88	1.80	1.92	1.83	1.86

Total_interspersed_repeats	NA	NA	NA	NA	NA	NA
Small_RNA	3.11	2.08	1.98	2.14	2.03	2.07
Satellites	0.04	0.03	0.03	0.02	0.03	0.01
Simple_repeats	27.75	19.54	18.94	18.39	19.50	19.31
Low_complexity	3.06	2.22	2.20	2.02	2.29	2.27

Figure 1. Comparison of number of MGEs between set T and set N. The y- axis represents the number of MGEs identified and the x- axis represents the different class of MGEs.

Figure 2. Comparison of percentage of sequence covered by MGEs between set T and set N. The y- axis represents the different class of MGEs and the x- axis represents the sequence covered by each class of MGE (in percentage).

Figure 3. Comparison of sequence length covered by MGEs between set T and set N. The y- axis represents the different class of MGEs and the x- axis represents the sequence covered (in base pairs) by each class of MGE.