Chapter

The Role of Introns for the Development of Inflammation-Mediated Cancer Cell

Begum Rokeya, Mohammad Asrafuzzaman, Maliha Tabassum Rashid and Shaeri Nawar

Abstract

Cancer and inflammation are connected by intrinsic pathways and extrinsic pathway where the intrinsic pathway is activated by genetic events including mutation, chromosomal rearrangement or amplification, and the inactivation of tumor-suppressor genes, as well as the extrinsic pathway, is the inflammatory or infectious conditions that increase the cancer risk. On the other hand, introns are non-coding elements of the genome and play a functional role to generate more gene products through splicing out, transcription, polyadenylation, mRNA export, and translation. Moreover, introns also may act as a primary element of some of the most highly expressed genes in the genome. Intron may contain their regulatory function as CRISPR system which is activated after the demand of specific gene for specific protein formation where those are required for gene expression, they go for transcription and rest of them form splicing. This chapter will focus on the plausible role of introns to influence the genetic events of inflammation-mediated cancer cell development.

Keywords: inflammation, cancer, intron retention, CRISPR, transcription, PcGs

1. Introduction

The functioning links between cancer and inflammation was approached by a great scientist and physician Rudolf Ludwing Carl Virchow in 1863 [1–3]. Thereafter, for long Virchow's idea had almost been unevaluated and discussed insufficiently [1]. Balkwill and colleagues (2001) supported Virchow's idea and stated that if molecular deregulation is the "match that lights the fire" of cancer, then some types of inflammation may act as the fuel that stimulates the flame. For instance, the inflammatory process act as a cofactor in malignancy in the bladder, cervical, ovarian, gastric, MALT (mucosa-associated lymphoid tissue) lymphoma, esophageal, colorectal, hepatocellular, bronchial, mesothelioma, and Kaposi sarcoma [3].

Currently, it is scientifically proven that inflammation promotes all stages of tumor formation as well as the development of cancer where chronic inflammation or non-resolving inflammation is playing a principal role in the initiation, promotion, malignant transformation, invasion and metastasis of cancer [4–9]. Interestingly, cancer-related inflammation is representing its 7th position as a

cancer hallmark and catching the current research attention in human cancer biology [5]. Basically, Inflammation act on cancer development by linking extrinsic and intrinsic pathway [9]. The extrinsic pathway develops inflammatory condition or microenvironment by inflammatory leukocytes particularly macrophages and soluble mediators (vasoactive amines such as histamine and serotonin, peptide such as bradykinin, and eicosanoids such as thromboxanes, leukotrienes, and prostaglandins) that raises cancer risk [9–11]. Intrinsic pathway is driven by genetic events (e.g. oncogenes, genetic aberrations) causing neoplastic transformation, initiate the expression of inflammation-related programs which guide the construction of an inflammatory microenvironment [11]. Inflammatory system involves the dynamic regulations of hundreds of genes and complex transcriptional program [12]. Moreover, the gene regulations by intron are often expressed in most of the cell through the effects of splicing or specific features [13–15]. Recently, several scientific reports claim that retained intron deregulates splicing machine in tumor transcriptoms [16–18]. Intron retention is considered as mis-splicing in which rather than being spliced out intron stays back and retained in mature mRNA [17]. However, the broad gap exists in monitoring of introns role in understanding of gene expression which could be a powerful tool in biotechnological and therapeutic applications. This chapter will cover intron's role in the development of inflammation, intron retaining genes causing inflammation to cancer and finally unfolding of a hypothesis about CRISPR like machine to monitor introns function.

2. Inflammation to cancer

2.1 Extrinsic pathway

Inflammation is activated by leukocytes which make inflammatory mediators in the extrinsic pathway and this pathway is also triggered by various infections and toxic agents such as gastric acid reflux, autoimmune disease etc. [9]. Patients with inflammatory bowel diseases have an increased chance of getting colorectal cancer. As an example, about 43% of patients with ulcerative colitis develop colorectal cancer [19]. Moreover, *in vivo* and *in vitro* experiments have shown that DNA can be damaged by reactive oxygen species (ROS) and nitrogen intermediates which are known as inflammation generated mediators [20, 21]. For example, the enzymes of nitrogen production (iNOS) are overly expressed in many cancer cells [22]. It has been shown scientifically that over expressed iNOS involves in free radical-mediated DNA damage as well as creates an inflammatory microenvironment [23–27]. In a hypoxic atmosphere, a heterodimeric transcription factor known as HIF-1 (HIF-1α & HIF-1β) binds to hypoxia regulated genes and triggers the activation of iNOS and vascular endothelium growth factor (VEGF) [28, 29]. Thus hypoxia-responsive molecules such as HIF-1α & HIF-1β play an integral role in tumor and cancer development [30]. Matsumoto et al., 2007 have shown that *Helicobacter pylori* produce cytidinedeaminase (AID) in gastric epithelium which induce chronic inflammation mediated cholangiocarcinoma [31, 32].

2.2 Intrinsic pathway

Intrinsic pathway is activated by genetic variation such as proto-oncogene activation, inactivated tumor suppressor genes, and chromosomal multiplication as well as mutation which develops neoplasia [22]. A gene coding protein namely tyrosine kinase RET shows rapid and ample genetic variation in human papillary thyroid carcinoma (PTC) and initiates the transcriptional program that links to the

development of inflammation [33]. Transcriptome profile is activated by tyrosine kinase RET in human papillary carcinoma and comprises with colony-stimulating factors (CSFs), interleukin 1β (IL-1β), cyclo-oxygenase 2 (COX2), chemokines attacking monocytes and dendritic cells (CCL2 & CCL20), angiogenicchemokines (CXCL8), matrix-degrading enzymes and inhibitors, chemokine receptor (CXCR4) [34]. For example, patients with lymph nodes metastasis have shown an elevated level of tyrosine kinase RET activated inflammatory molecules in their biopsy results which demonstrate that genetic events have taken place in the pathogenesis of tumor and constructed an inflammatory environment [33–35]. Moreover, *Ras* oncogenes were known to be the most dominant genes that tend to get mutated rapidly and play a significant role in tumorigenesis. *Ras* oncogenes family includes KRAS, HRAS, and NRAS and their mutation has been observed in 25–30% of tumor specimens which has an impact on the KRAS locus [22]. For instance cervical cancer has shown that shifting of *RAS* oncogenes makes a chemokine named CXCL8 which participates in tumor development [36, 37]. Furthermore, polycomb complex target genes (PcGs) play a salient role in the growth of the embryo and aging through epigenetic rearranging. These groups of genes also involve in abnormal DNA methylation and histone modification in cancer cells [38]. As an example, Yu H and colleagues (2007) reported that a mouse model with intestinal inflammation and cancer shows abnormal DNA methylation where over 70% of abnormal methylated genes were observed in PcGs [38].

3. Functional role of intron for development of cancer

Intron is defined as any intervening nucleotide sequence that formed splicing at the RNA level [39]. Intron was first discovered in 1970s with a traditional views that the coding region of eukaryotic genes are interrupted by introns which are spliced out from pre mature mRNA transcripts before the formation of mature mRNA [39–41]. After the elucidation of intron splicing mechanism, scientist became excited about its function on gene expression and speculated that may be introns carry out some function like regulation of splicing function, regulation of transcription, evolutionary function or coding capacity but there was no clear examples of their active functions on gene expression [41]. From the starting 21st century, many researches have claimed about the intron function on gene expression or intron mediated enhancement of gene expression [13, 42–47]. However, a question remains unclear that within the genome, who is responsible to remember or decide which intron or parts of nucleotide sequences are necessary to stay within the mature mRNA stand for inflammatory gene expression rather than form splicing that result in cancer? Current chapter sheds light on the above question and hypothesize CRISPR like machine in perspective of inflammation mediated cancer development.

After the discovery of alternative splicing (AS), the transcriptomic and proteomic complexity has increases significantly [47, 48]. Recent breakthrough studies in high-throughput sequencing have explored a pivotal role of AS in normal biology that more than 95% of human multi exonic genes are subject to AS and produce at least two alternative isoforms [49, 50]. Moreover, Braunschweig and colleague compared 11 vertebrate species and observed that about 50–75% of multi-exonic genes are affected by intron retention (IR) which is one kind of AS [47, 48, 51]. While another study showed that IR affects near about 80% of protein coding genes in humans [52]. Some scientific reports also considered IR as a harmful process for the body by slowing down splicing kinetics and delaying the onset of gene expression, by raising pre-mRNA degradation in the nucleus through nuclear exosomes and finally by enhancing cytoplasmic pre-mRNA degradation through nonsense-mediated decay [51, 53, 54].

This statement is also supported by the Green and colleague's research as the genes that encoded the regulators of macrophage transcription, signaling inflammation, and phagocytosis has increased their expression when the IR events decreased [55]. As it is known, that intron retention (IR) is the process where instead of typically being spliced out, the introns remain intact in the mature mRNAs and thus whole process of IR supposedly has numerous physiological drawbacks resulting in different diseases [47, 48]. Currently, many researchers strongly claim that IR is a key mechanism to control gene expression during the development, differentiation and activation of several types of mammalian cell [56–63]. A recent study by Green and colleague claimed that intron retention affected the expression of key genes *(ID2*, *IRF7*, *ENG*, and *LAT*) involved in the development and function of macrophages those are the key inflammatory regulator [55]. So IR gene might be acting as one of the major causes of inflammation mediated cancer development (**Table 1**).

3.1 *TGIF2* gene

PCR technique revealed two alternate splice forms of *TGIF2* gene that were found in mice. The coding sequence of *TGIF2* gene both in human and mice consists with intron that is retained [78]. The splicing phenomenon depends on the *TGIF2* coding sequence where both splice forms encoded for an active transcriptional repressor protein that is used to repress independent TGFβ and dependent TGFβ transcription [79]. Moreover, Melhuish and colleague have shown that the transcriptional core-repressor mSin3 interacts with human and mice*TGIF2* and also revealed that the *TGIF2* gene retains intron at a negligible amount which is not spliced out from human mRNA but a bigger amount is spliced out from the mice and thus correlated with variant cancers [79]. These findings are also supported by the study of Imoto et al., 2020 where the authors claimed that amplification and over-expression of *TGIF2* gene lead to ovarian cancer [80]. The *TGIF2* gene is located in chromosome 20q11.2–12 [80]. Often in solid human tumors, it has been observed that the long arm of chromosome 20 is highly amplificated [64]. Rapid amplification of *TGIF2* in chromosome 20 leads to ovarian cancer which is observed in the cell lines of ovaries [80]. It is known that inflammation helps to grow tumor as well as development of cancer [4]. So the *TGIF2* gene might have induced inflammation during the tumourigenesis later on which turned into ovarian cancer.

3.2 *EBNA-3* gene

EBNA-3 gene involves in multiple regulation during their expression due to IR retention. For instance, a study by Kienzle et al., (1999) showed that a stop codon might be inserted into a frame shift because of IR and thus the translation process could be stopped as a premature termination of *EBNA-3* gene [81]. Moreover, it was depicted that IR can change the expression pattern of the *EBNA-3* gene in human B-lymphocyte where its protein plays a pivotal role in cell proliferation and transformation. They also play a censorious part in the development of lymphoma [82]. Patients with chronic inflammation because of autoimmune disorder have a higher risk to develop lymphoma [83]. So, herein could be a strong possibility to influence inflammation mediated lymphoma development by *EBNA-3* gene.

3.3 *APOE4*

The expression of the *APOE4* isoform shows a relation between intron retention and Alzheimer's disease (AD). The prevalence of AD was longitudinally associated with a reduced risk of cancer where the cancer incidence was associated with

Gene name	Protein name	General function	Types of cancer that is developed by IR gene	Abnormal function	References
TGIF2	TGFB Induced Factor Homeobox 2	TGF-β-induced factor homeobox 2 (*TGIF2*) is known to be transcription regulator that plays significant role in the regulation of development and cell fate decisions. Abnormal expression of TGIF family proteins has been noticed in numerous cancers which include ovarian, esophageal, and colorectal cancers.	Ovarian cancer	Over expressed	[64, 65]
EBNA-3	Epstein–Barr nuclear antigen 3	This gene plays a principle role for activation and immortalization of human B-cells. Represses transcription of viral promoters TP1 and Cp interact with RBPJ and also inhibits the *EBNA2*-mediated activation of these promoters. As Cp is known to be the promoter for all the *EBNA* mRNAs, *EBNA3A* probably provides a negative autoregulatory control loop.	Lymphoma and Epithelial cancers	Amplication	[66, 67]
APOE4	Apolipoprotein E4	This gene gives instructions to make a protein called apolipoprotein E. This protein combines with fats (lipids) in the human body to form molecules called lipoproteins. Lipoproteins in body package cholesterol and other fats and transfer them by the bloodstream.	Breast cancer	Increased frequency	[68, 69]
EGFR	Epidermal growth factor receptor	Receptor tyrosine kinase binding ligands of the EGF family. They activate a lot of signaling cascades to transform them extracellular cues into appropriate cellular responses.	Lung cancer	Mutation or cell damage	[70, 71]
ROS1	Receptor Tyrosine Kinase	*ROS1* is a proto-oncogene which encodes a type I integral membrane protein with receptor tyrosine kinase (RTK) activity. It is a member of the insulin receptor family and it is also involved in downstream signaling processes in cell growth and differentiation.	Ovarian cancer, cholangiocarcinoma, inflammatory myofibroblastic tumor, colorectal, and angiosarcoma	Mutation	[72, 73]

Gene name	Protein name	General function	Types of cancer that is developed by IR gene	Abnormal function	References
RUNX1	RUNX Family Transcription Factor 1	The protein encoded by this gene represents the alpha subunit of CBF(Core binding factor) and is thought to be involved in the development of normal hematopoiesis. Chromosomal translocations involving by this gene are well-documented and have been associated with several types of leukemia. Three transcript variants encoding different isoforms have been found for this gene	Myeloid and lymphoid	Mutation	[74, 75]
TP53	Tumor Protein 53	*TP53* gene gives instructions for making a protein called tumor protein p53. This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing (proliferating) too fast or in an uncontrolled way	Breast cancer, bone and soft tissue sarcomas, brain tumors and adrenocortical carcinomas (ADC), leukemia, stomach cancer and colorectal cancer	Gene Alternation or Deletion or Mutation	[76, 77]

Table 1.
List of intron retention genes and their functions.

a reduced risk of AD [84]. Significant scientific evidence has shown that *APOE4* could able to aggravate more neurodegeneration, tau pathology and inflammation [85–87]. Liestøl et al., 2000 have observed that genotypes of *APOE4* increases the risk of cancer in patients with immunodeficiency. Interestingly, 24.6% of *APOE4* alleles have been found in variant cancer cases where 13.5% were found in noncancerous cases [88]. As the higher frequency of *APOE4* increases the risk of cancer, it also worsens inflammation. It might be possible that *APOE4* may initiate inflammation and by making it worse, it causes cancer in people with immunodeficiency.

3.4 *EGFR, ROS1, RUNX1*

It was claimed that 2340 and 1422 genes show tumor-specific and normal tissue-specific retention events respectively [89, 90]. For example, *EGFR, ROS1, RUNX1* play salient roles in carcinogenesis [72, 89]. *ROS1* gene fusion has been observed in a substantial number of malignancies which comprises ovarian cancer, cholangiocarcinoma, inflammatory myofibroblastic tumor, colorectal, and angiosarcoma [72]. Abnormal *EGFR* expression can initiate different types of respiratory diseases such as inflammation mediated lung fibrosis, cancer, and multiple hypersecretory diseases comprising COPD, asthma and cystic fibrosis [91]. Furthermore, *RUNX1* mutation has been observed in numerous malignancies in myeloid and lymphoid cell [92]. Bellissimo et al., 2020 showed that *RUNX1* regulates the signaling pathways of TLR1/2 and TLR4 and with the help of neutrophils it can produce inflammatory cytokines as well as develop inflammation mediated leukemia [74].

3.5 *TP53*

Intron retention frequency may be responsible to inactive tumor suppressor genes in many cancers cell [93]. Study revealed that in cancerous condition, retained intron transcript exits in NMD pathway and inactivates the *TP53* gene. Additionally, *TP53* gene provides instruction to code for a protein named p53 which can produce pro-inflammatory cytokines [92]. Mutation of p53 can cause inflammation mediated cancer [94].

4. Phylogenetic tree analysis of IR gene, PcGs and Ras oncogene

The evolutionary history and relationship of an organism or group of species are named phylogeny. Phylogeny depicts the connection of an organism. Phylogenetic relationships give information on shared common ancestry but not obligatory how organisms are similar or different. Phylogenic tree analysis of all genes those are performing intron retention scenario, PcGs and Ras oncogenes (**Figures 1**–**3**) has

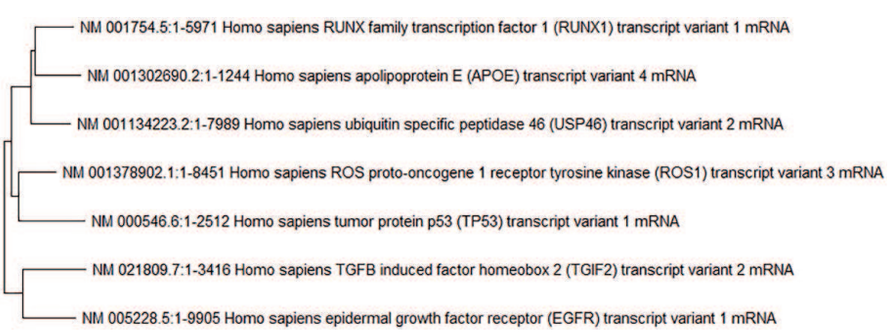

Figure 1.
Phylogenetic tree of intron retaining (IR) genes.

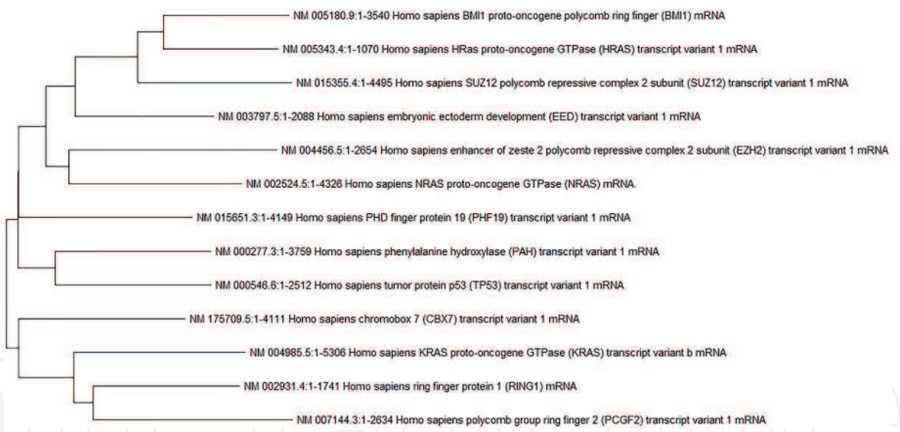

Figure 2.
Phylogenic tree between RAS oncogenes and PcGs.

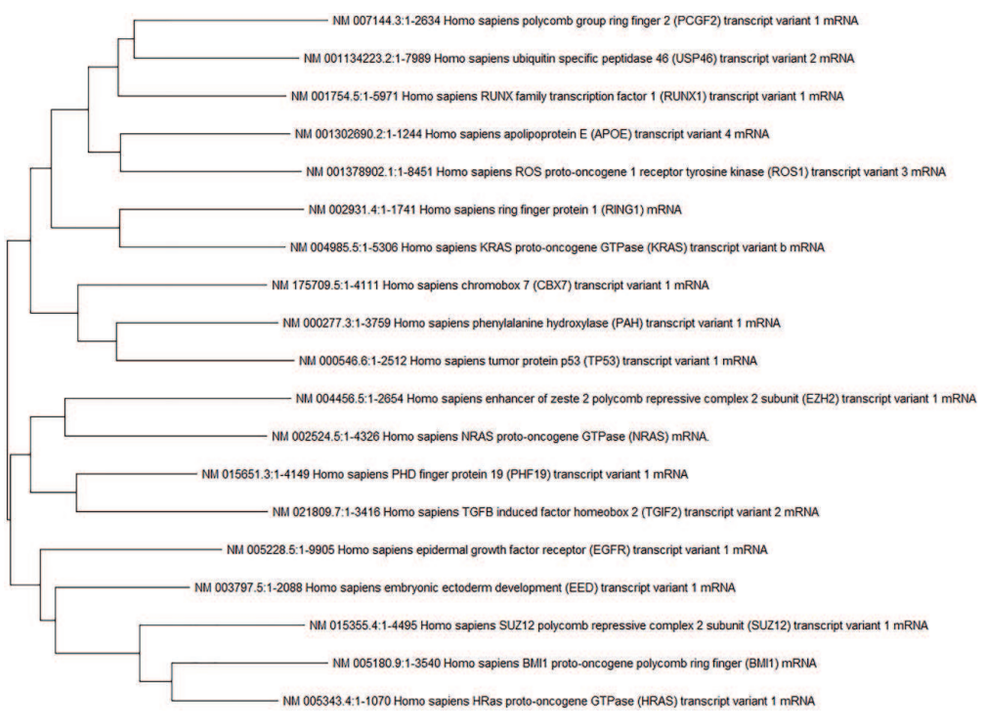

Figure 3.
Phylogenic tree among IR gene, RAS oncogenes and PcGs.

done to find out the relation among the genes. MEGA X software has been used to construct the phylogenic tree to understand the relation among all the cancers genes (**Tables 1** and **2**).

Figure 1 demonstrates that the vertical line on the left side is the common ancestor or the root of the seven gene sequences from which the genes have been evolved in a period. The evolution period can be explained through the horizontal lines near the sequences. The small length of lines before sequences means the sequences evolved in a short period whereas the long length of lines means longer time was needed for the sequences to be evolved. The common ancestor or root has been divided into two branch points. From the first branch point total of five sequences have been modified which were *RUNX1, APOE4, EBNA-3(USP46), ROS1,* and *TP53.* From the second branch point, two sequences have been evolved *TGIF2* and *EGFR.* The phylogenetic tree has three separate clades. Clade means a variety of species that include all the descendants of a common ancestor. In the first clade, the first two sequences of genes *RUNX1* and *APOE4* are closer to the ancestor than *EBNA-3* as they are from the same node. In the second clade, among *ROS1* and

Gene name	Protein name	General function	Types of cancer that is developed by IR gene	Abnormal function	References
BMI 1	B-lymphoma Moloney murine leukemia virus insertion region-1	This gene functions through chromatin remodeling as a principle epigenetic repressor of numerous regulatory genes involved in embryonic development and self-renewal in somatic stem cell and also plays a median role in DNA damage repair. It is an oncogene and abnormal expression is related with multiple cancers and resistance to certain chemotherapies.	Gastric, ovarian, breast, head and neck, pancreatic and lung cancer, primary hepatocellular carcinoma (HCC) and endometrial carcinoma	Over expression	[95, 96]
CBX7	Chromobox proteins 7	This gene encodes a protein that comprises the CHROMO (CHRomatin Organization MOdifier) domain. It is thought to control the lifespan of several normal human cells.	Breast, Thyroid, Colorectal, Pancreas, Lung carcinoma and Glioblastoma	Down regulation	[97, 98]
PH	Phenylalanine Hydroxylase	This gene gives instructions for making an enzyme called phenylalanine hydroxylase. This enzyme is responsible for the primary step in processing phenylalanine, which is a building block of proteins (an amino acid) obtained through the diet.	Liver cancer	Down regulation	[99, 100]
RING 1	Ring Finger 1A	This gene encodes proteins characterized by a RING domain, a zinc-binding motif related to the zinc finger domain. The gene product can bind DNA and can act as a transcriptional repressor. It is related with the multimericpolycomb group protein complex.	Hepatocellular and colorectal carcinomas	Down regulation	[101, 102]
MEL18	Polycomb group ring finger 2	*Mel-18* functions as a tumor suppressor via downregulation of *BMI1*. Single Nucleotide Polymorphism and down regulation of *Mel-18* is associated with prostate cancer.	Breast cancer, Prostate cancer	Loss of expression and down regulation	[103, 104]
EZH2	Enhancer of zeste homolog-2	The *EZH2*gene makes an enzyme methyltransferase. Histone methyltransferases modify proteins called histones, which are structural proteins that bind to DNA and shape chromosomes. Addition of a molecule (methyl group) to histones (methylation), histone methyltransferases can turn off (suppress) the activity of certain genes, an essential process in normal development.	Breast cancer, Colorectal cancer, Endometrial cancer, Gastric cancer, Liver cancer, Lung cancer	Over expression	[105, 106]

Gene name	Protein name	General function	Types of cancer that is developed by IR gene	Abnormal function	References
EED	Embryonic ectoderm development	This gene encodes a member of the Polycomb-group family. It maintains the transcriptional repressive state of genes over successive cell generations. This protein interacts with enhancer of zeste 2, the cytoplasmic tail of integrin beta7, immunodeficiency virus type 1 (HIV-1) MA protein, and histone deacetylase proteins. This protein mediates repression of gene activity through histone deacetylation, and may act as a specific regulator of integrin function. Two transcript variants encoding distinct isoforms have been identified for this gene.	Colorectal Cancer, acute myeloid leukemia and diffuse large B cell lymphoma	High expression	[107–109]
SUZ12	suppressor of zeste 12 homolog	This zinc finger gene has been detected at the breakpoints of a recurrent chromosomal translocation reported in endometrial stromal sarcoma. Recombination of these breakpoints results in the fusion of this gene and *JAZF1*. The protein encoded by this gene comprises a zinc finger domain in the C terminus of the coding region	Colorectal, ovarian and non-small lung cancer, head and neck squamous cell carcinoma	Over expression	[110, 111]
PCL3	PHD fing protein 19	*PHF19* promotes the proliferation, migration, and chemosensitivity of glioblastoma to doxorubicin through modulation of the SIAH1/beta-catenin axis. Human *PCL3* has an oncogenic role in hepatocellular carcinoma by activating the beta-catenin/IL6 signaling axis to promote metastasis	Hepatocellular carcinoma, glioma, and ovarian cancers, glioblastoma progression, prostate cancer	Over expression	[112, 113]
TP53	TumourProtein 53	Functions are the same as discussed in **Table 1**	Types of cancers are the same as discussed in **Table 1**	Gene alternation or deletion or mutation	[76, 77]
KRAS	Kirsten rat sarcoma viral oncogene	The *KRAS* gene gives instructions for making a protein called *K-Ras* which is a part of a signaling pathway known as the *RAS/MAPK* pathway. The protein relays signals from outside the cell to the cell's nucleus	Non-small cell lung cancer, colorectal cancer, and pancreatic cancer	Mutation	[114, 115]

Table 2.
List of PcGs genes and their functions.

TP53, ROS1 is closer to the root as its horizontal line is smaller than the *TP53* ones. In the third clade, *EGFR* is much closer to the root than *TGIF2* as its horizontal line is smaller and close to the ancestor than *TGIF2.*

Figure 2 depicts that both type of cancer genes (*RAS* oncogene and PcGs) were evolved from a common root or ancestor. The tree demonstrates that *BMI 1, HRAS, SUZ12,* and *EED* had been sharing the most recent ancestor but *BMI 1, HRAS,* and *SUZ12* remain closer than *EED* as they share the same root. Interestingly, *BMI 1 & EZH2* from *PcGs* and *HRAS & NRAS* from *Ras* oncogene are closely related to each other respectively where they are from different group's gene. The following three genes *PHF19/PCL3, PH* and *TP53* were from the same clade and they are near to one another. The last four genes *CBX7, KRAS, RING 1* and *MEL 18(PCGF2)* were from the same clade as they share the most common ancestor.

Figure 3 demonstrates that all the genes were evolved from a common ancestor. The IR gene *APOE 4* and *ROS1* are closely related each other as expected. However interestingly, IR gene *EBNA-3*shares the common ancestor with *PCGF(MEL18).* So herein might be chances for *MEL18* or *PCGF2* gene to develop inflammation mediated cancer by the influencing of IR.

5. Hypothesis: CRISPR like function might be a functioning model of intron

CRISPR-Cas9 is also known as a genome-editing tool where CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats and Cas9 is an enzyme that cuts foreign DNA [116]. In the 21st century, CRISPR-Cas9 is being used immensely in medical technology to edit, remove or add a gene to correct genetic defects [116]. CRISPR has conformed from the natural defense mechanism of bacteria, archaea and developed an immune system by CRISPR loci [116]. CRISPR and Cas9 enzyme serve as an immune guard and provide safety against bacteriophage, viruses, and foreign invaders [117–119]. The immunization process after invading foreign genetic elements, a small fragments of foreign DNA are integrated into the CRISPR repeat-spacer array within the host chromosome as new spacers. Thus, a genetic record of prior infection will save into the host body that enables to prevent future invasion of the same invader [120, 121] (**Figure 4**).

The nucleotide repeats and spacers are main two component of CRISPR. Repeated sequence of nucleotide is distributed in the CRISPR region and Spacers are

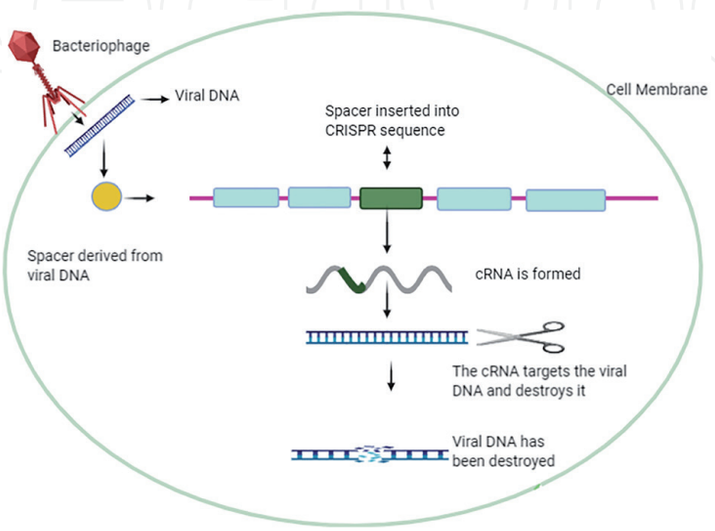

Figure 4.
CRISPR biology.

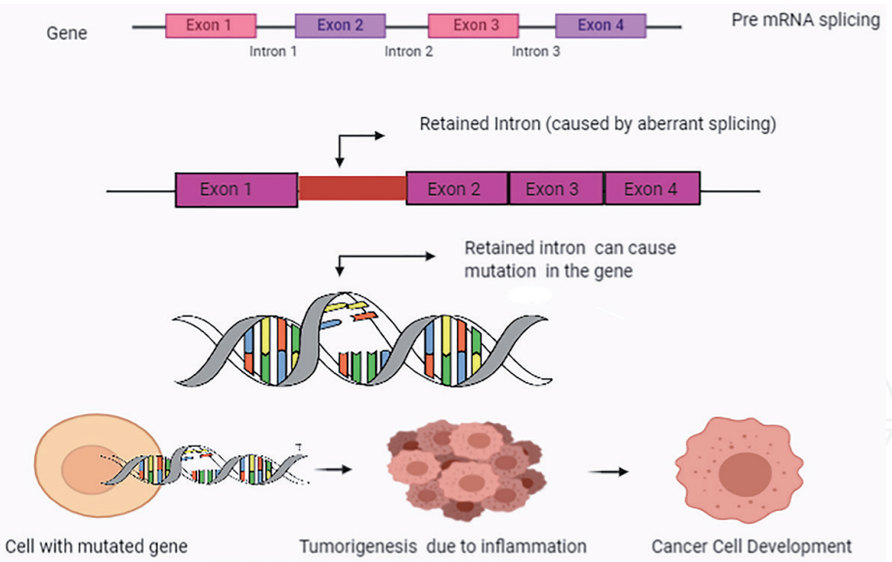

Figure 5.
Illustration of retained intron in the gene causing mutation which leads to inflammation and tumorigenesis resulting in development of cancer.

a small portion of DNA present in the CRISPR region. Destruction of foreign invading DNA or RNA occurs by Cas9 enzyme. If in future, the foreign body again attacks the organism, they fight it off as they have the virus or foreign invader's DNA from beforehand and thus they recognize it and kill it [121]. So in CRISPR biology, spacer acts as a responsible sequence to remember or decide which foreign body needs to be killed where Cas9 enzyme plays a pivotal role. According to our hypothesis, intron network might work as CRISPR like functioning model.

According to the section 3, it is confirmed that IR have the ability to influence inflammation mediated cancer development. From the CRISPR function it is clear that according to the demand of the cell, CRISPR can get activated. It might be possible that according to the demand of the abnormal cells, intron retention may form to confirm inflammation mediated malignancy state (**Figure 5**). Moreover, our phylogenetic tree analysis depicts that PcGs, *RAS* oncogenes, and intron retaining genes are related to each other and they all share a common ancestor. As all three categories of genes initiate cancer development in humans, it might be possible that PcGs and RAS oncogenes can express themselves as intron retaining genes or vice versa.

6. Conclusions

Cancer is a genetic disease and is one of the leading causes of death around the world. As a genetic event, the intron retention causes inflammation as well as the development of cancer cells. So far, it is clear that the intron is spliced away during gene expression while exons remain and express the genes. However, the retention of introns is an unlikely phenomenon that differs from the common hypothesis and appears anomalous. The genes involved in retaining intron have a carcinogenic effect. It may be speculated that some nucleotide sequence whether it is coding or non-coding region could function as a memory sequence, hence are able to remember intronic sequence as like spacer responsibility of CRISPR system and would only be functioning according to the demand of the cell. Future in depth analysis on intron retaining genes is required to explore their effect on inflammation and cancer.

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Appendices and nomenclature

VEGF	Vascular endothelium growth factor
PTC	Papillary thyroid carcinoma
CSFs	colony-stimulating factors
IL-1β	Interleukin 1β
COX2	Cyclo-oxygenase 2
PcGs	Polycomb complex target genes
AD	Alzheimer's disease
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
AS	Alternative splicing
IR	Intron retention
COPD	Chronic obstructive pulmonary disease
MALT	Mucosa-associated lymphoid tissue

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References

[1] Schmidt A, Weber OF: In Memoriam of Rudolf Virchow: A Historical Retrospective Including Aspects of Inflammation, Infection and Neoplasia. Contributions to Microbiology. 2006; 1-15. Doi: 10.1159/000092961

[2] Balkwill F, Mantovani A: Inflammation and cancer: back to Virchow?. Lancet (London, England). 2001;357(9255):539-545. DOI: https://doi.org/10.1016/S0140-6736(00)04046-0

[3] Balkwill F: Tumor necrosis factor or tumor promoting factor?. Cytokine Growth Factor Rev. 2002);13, (2):135-141. DOI: 10.1016/s1359-6101(01)00020-x

[4] Greten FR, Grivennikov SI: Inflammation and Cancer: Triggers, Mechanisms, and Consequences. Immunity. 2019;51(1):27-41. DOI:https://doi.org/10.1016/j.immuni.2019.06.025

[5] Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A: Cancer-related inflflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30:1073-1081. DOI: 10.1093/carcin/bgp127

[6] Todoric J, Antonucci L, Karin M: Targeting Inflammation in Cancer Prevention and Therapy. Cancer Prevention Research . 2016;9(12):895-905. DOI: 10.1158/1940-6207

[7] Fishbein A, Wang W, Yang H, Yang J, Hallisey VM, Deng J, Verheul SML, Hwang SH, Gartung A, Wang Y, Bielenberg DR, Huang S, Kieran MW, Hammock BD, Panigrahy D: Resolution of eicosanoid/cytokine storm prevents carcinogen and inflammation-initiated hepatocellular cancer progression. Proceedings of the National Academy of Sciences. 2020;117(35):21576-21587. DOI: https://doi.org/10.1073/pnas.2007412117

[8] Song XD, Wang YN, Zhang A, Liu B: Advances in research on the interaction between inflammation and cancer. Journal of International Medical Research. 2019;48(4). DOI: https://doi.org/10.1177/0300060519895347

[9] Del Prete A, Allavena P, Santoro G, Fumarulo R, Corsi MM, Mantovani A: Molecular pathways in cancer-related inflammation. Biochemia Medica. 2011;264-275. DOI: https://doi.org/10.11613/bm.2011.036

[10] Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezmee MNM: The crucial roles of inflammatory mediators in inflammation: A review. Veterinary World. 2018;11(5):627-635. DOI: https://doi.org/10.14202/vetworld.2018.627-635

[11] Allavena P, Garlanda C, Borrello MG, Sica A, Mantovani A: Pathways connecting inflammation and cancer. Current Opinion in Genetics & Development. 2008;18(1):3-10. DOI:https://doi.org/10.1016/j.gde.2008.01.003

[12] Smale ST, Natoli G: Transcriptional Control of Inflammatory Responses. Cold Spring Harbor Perspectives in Biology. 2014;6(11). DOI: https://doi.org/10.1101/cshperspect.a016261

[13] Rose AB: Introns as Gene Regulators: A Brick on the Accelerator. Frontiers in Genetics. 2019;9:1-6. DOI: https://doi.org/10.3389/fgene.2018.00672

[14] Chorev M, Carmel L: The Function of Introns. Frontiers in Genetics. 2012;3:1-15. DOI: https://doi.org/10.3389/fgene.2012.00055

[15] Zhang D, Hu Q, Liu X, Ji Y, Chao H.-P, Liu Y, Tracz A, Kirk J,

Buonamici S, Zhu P, Wang J, Liu S, Tang DG: Intron retention is a hallmark and spliceosome represents a therapeutic vulnerability in aggressive prostate cancer. Nature Communications. 2020;11(1):1-19. DOI: https://doi.org/10.1038/s41467-020-15815-7

[16] Smart AC, Margolis CA, Pimentel H, He MX, Miao D, Adeegbe D, Fugmann T, Wong KK, Van Allen EM: Intron retention is a source of neoepitopes in cancer. Nature Biotechnology. 2018;36(11): 1056-1058. DOI: https://doi.org/10.1038/nbt.4239

[17] Dvinge H, Bradley RK: Widespread intron retention diversifies most cancer transcriptomes. Genome Med. 2015;7:45. DOI: https://doi.org/10.1186/s13073-015-0168-9

[18] Jung H, Lee D, Lee J, Park D, Kim YJ, Park WY, Hong D, Park PJ, Lee E: Intron retention is a widespread mechanism of tumor-suppressor inactivation. Nature Genetics. 2015;47(11):1242-1248. DOI:https://doi.org/10.1038/ng.3414

[19] Ferrone C, Dranoff G: Dual roles for immunity in gastrointestinal cancer. J Clin Oncol. 2010;28(26):4045-4051. DOI:10.1200/JCO.2010.27.9992

[20] Wu Y, Zhou BP: TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. Br J Cancer. 2010;102(40):639-644. DOI:10.1038/sj.bjc.6605530

[21] Hold GL, El-Omar ME: Genetic aspects of inflammation and cancer. Biochemical Journal. 2008;410(2):225-235. DOI: https://doi.org/10.1042/bj20071341

[22] Multhoff G, Molls M, Radons J: Chronic Inflammation in Cancer Development. Frontiers in Immunology. 2012;2:1-17. DOI: https://doi.org/10.3389/fimmu.2011.00098

[23] Kawanishi S, Hiraku Y, Pinlaor S, Ma N. Oxidative and nitrative DNA damage in animals and patients with inflflammatory diseases in relation to inflammation-related carcinogenesis. Biol Chem . 2006;387(4):365-372. DOI: 10.1515/BC.2006.049

[24] Lechner M, Lirk P, Rieder J: Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin.Semin Cancer Biol . 2005 Aug;15(4):277-289. DOI:10.1016/j.semcancer.2005.04.004

[25] Ohshima H: Genetic and epigenetic damage induced by reactive nitrogen species: implications in carcinogenesis. Toxicol Lett. 2003;140-141:99-104. DOI: 10.1016/s0378-4274(02)00506-4

[26] Ohshima H, Tazawa H, Sylla BS, Sawa T: Prevention of human cancer by modulation of chronic inflflammatory processes. Mutat Res. 2005 ;591(1-2):110-122. DOI:10.1016/j.mrfmmm.2005.03.030.

[27] Jaiswal M, LaRusso NF, Gores GJ: Nitric oxide in gastrointestinal epithelial cell carcinogenesis: linking inflflammation to oncogenesis. Am J Physiol Gastrointest Liver Physiol. 2001;281(3):G626-G634. DOI: 10.1152/ajpgi.2001.281.3.G626.

[28] Jain RK: Tumor angiogenesis and accessibility: role of vascular endothelial growth factor. Semin Oncol. 2002;29:3-9. DOI:10.1053/sonc.2002.37265

[29] Semenza GL: Targeting HIF-1 for cancer therapy. Nat Rev Cancer. 2003;3(10):721-732. DOI: 10.1038/nrc1187.

[30] Kimbro KS, Simons JW: Hypoxia-inducible factor-1 in human breast and prostate cancer. Endocr Relat Cancer. 2006;13(3):739-749. DOI: 10.1677/erc.1.00728

[31] Matsumoto Y, Marusawa H, Kinoshita K, Endo Y, Kou T, Morisawa T,

Azuma T, Okazaki IM, Honjo T, Chiba T: Helicobacter pylori infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nat Med. 2007;13(4):470-476. DOI: 10.1038/nm1566

[32] Komori J, Marusawa H, Machimoto T, Endo Y, Kinoshita K, Kou T, Haga H, Ikai I, Uemoto S, Chiba T: Activation-induced cytidine deaminase links bile duct inflammation to human cholangiocarcinoma. Hepatology. 2008;47(3):888-896. DOI: 10.1002/hep.22125

[33] Borrello MG, Alberti L, Fischer A, Degl'innocenti D, Ferrario C, Gariboldi M, Marchesi F, Allavena P, Greco A, Collini P, Pilotti S, Cassinelli G, Bressan P, Fugazzola L, Mantovani A, Pierotti MA: Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. Proc Natl Acad Sci U S A. 2005;102(41):14825-14830. DOI:10.1073/pnas.0503039102

[34] Cerutti JM, Oler G, Michaluart PJ, Delcelo R, Beaty RM, Shoemaker J, Riggins GJ: Molecular profiling of matched samples identifies biomarkers of papillary thyroid carcinoma lymph node metastasis. Cancer Res. 2007;67(16):7885-7892. DOI: 10.1158/0008-5472

[35] De Falco V, Guarino V, Avilla E, Castellone MD, Salerno P, Salvatore G, Faviana P, Basolo F, Santoro M, Melillo RM: Biological role and potential therapeutic targeting of the chemokine receptor CXCR4 in undifferentiated thyroid cancer. Cancer Res. 2007;67(24):11821-11829. DOI:10.1158/0008-5472

[36] Masih-Khan E, Trudel S, Heise C, Li Z, Paterson J, Nadeem V, Wei E, Roodman D, Claudio JO, Bergsagel PL, Stewart AK: MIP-1alpha (CCL3) is a downstream target of FGFR3 and RAS-MAPK signaling in multiple myeloma. Blood. 2006;108(10):3465-3471. DOI:10.1182/blood-2006-04-017087

[37] Wang D, Wang H, Brown J, Daikoku T, Ning W, Shi Q, Richmond A, Strieter R, Dey SK, DuBois RN: CXCL1 induced by prostaglandin E2 promotes angiogenesis in colorectal cancer. J Exp Med. 2006;203(4):941-951.DOI:10.1084/jem.20052124

[38] Yu H, Kortylewski M, Pardoll D: Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol. 2007;7(1):41-51. DOI: 10.1038/nri1995

[39] Berget SM, Moore C, Sharp PA: Spliced segments at the 5' terminus of adenovirus 2 late mRNA. Proc Natl Acad Sci U S A. 1977;74(8):3171-3175. DOI: 10.1073/pnas.74.8.3171

[40] Chow LT, Gelinas RE, Broker TR, Roberts RJ: An amazing sequence arrangement at the 5' ends of adenovirus 2 messenger RNA. Cell. 1977;12(1):1-8. DOI; 10.1016/0092-8674(77)90180-5

[41] Long M, de Souza S J: Intron-exon structures. Advances in Genome Biology. 1998;143-178. DOI: https://doi.org/10.1016/s1067-5701(98)80020-x

[42] Baier T, Jacobebbinghaus N, Einhaus A, Lauersen KJ, Kruse O: Introns mediate post-transcriptional enhancement of nuclear gene expression in the green microalga Chlamydomonas reinhardtii. PLoS Genet. 2020;16(7):e1008944. DOI: 10.1371/journal.pgen.1008944

[43] Gallegos JE, Rose AB: An intron-derived motif strongly increases gene expression from transcribed sequences through a splicing independent mechanism in Arabidopsis thaliana. SciRep.2019;9(1):13777.DOI:10.1038/s41598-019-50389-5

[44] Jin Y, Fei M, Rosenquist S, Jin L, Gohil S, Sandström C, Olsson H,

Persson C, Höglund AS, Fransson G, Ruan Y, Åman P, Jansson C, Liu C, Andersson R, Sun C: A Dual-Promoter Gene Orchestrates the Sucrose-Coordinated Synthesis of Starch and Fructan in Barley. Mol Plant.2017;10(12):1556-1570. DOI:10.1016/j.molp.2017.10.013

[45] Akua T, Shaul O: The Arabidopsis thaliana MHX gene includes an intronic element that boosts translation when localized in a 5' UTR intron. J Exp Bot. 2013 ;64(14):4255-4270. DOI:10.1093/jxb/ert235

[46] Gromak N: Intronic microRNAs: a crossroad in gene regulation. Biochem Soc Trans. 2012;40(4):759-761. DOI:10.1042/BST20120023

[47] Kim MJ, Kim H, Shin JS, Chung CH, Ohlrogge JB, Suh MC: Seed-specific expression of sesame microsomal oleic acid desaturase is controlled by combinatorial properties between negative cis-regulatory elements in the SeFAD2 promoter and enhancers in the 5'-UTR intron. Mol Genet Genomics. 2006;276(4):351-368. DOI: 10.1007/s00438-006-0148-2.

[48] Liu Y, Gonzàlez-Porta M, Santos S, Brazma A, Marioni JC, Aebersold R, Venkitaraman AR, Wickramasinghe O: Impact of Alternative Splicing on the Human Proteome. Cell reports. 2017;20(5):1229-1241. https://doi.org/10.1016/j.celrep.2017.07.025

[49] Merkin J, Russell C, Chen P, Burge CB: Evolutionary dynamics of gene and isoform regulation in Mammalian tissues. Science. 2012;338(6114):1593-1599. DOI:10.1126/science.1228186

[50] Barbosa-Morais NL, Irimia M, Pan Q, Xiong HY, Gueroussov S, Lee LJ, Slobodeniuc V, Kutter C, Watt S, Colak R, Kim T, Misquitta-Ali CM, Wilson MD, Kim PM, Odom DT, Frey BJ, Blencowe BJ: The evolutionary landscape of alternative splicing in vertebrate species. Science. 2012;338(6114):1587-1593. DOI: 10.1126/science.1230612

[51] Braunschweig U, Barbosa-Morais NL, Pan Q, Nachman EN, Alipanahi B, Gonatopoulos-Pournatzis T, Frey B, Irimia M, Blencowe BJ. Widespread intron retention in mammals functionally tunes transcriptomes. Genome Res. 2014;24(11):1774-1786. DOI:10.1101/gr.177790.114

[52] Middleton R, Gao D, Thomas A, Singh B, Au A, Wong JJ, Bomane A, Cosson B, Eyras E, Rasko JE, Ritchie W. IRFinder: assessing the impact of intron retention on mammalian gene expression. Genome Biol. 2017;18(1):51. DOI: 10.1186/s13059-017-1184-4

[53] Niemelä EH, Oghabian A, Staals RH, Greco D, Pruijn GJ, Frilander MJ: Global analysis of the nuclear processing of transcripts with unspliced U12-type introns by the exosome. Nucleic Acids Res. 2014;42(11):7358-7369. DOI: 10.1093/nar/gku39

[54] Lejeune F, Maquat LE: Mechanistic links between nonsense-mediated mRNA decay and pre-mRNA splicing in mammalian cells. Curr Opin Cell Biol. 2005;17(3):309-315. DOI: 10.1016/j.ceb.2005.03.002

[55] Green ID, Pinello N, Song R, Lee Q, Halstead JM, Kwok CT, Wong ACH, Nair SS, Clark SJ, Roediger B, Schmitz U, Larance M, Hayashi R, Rasko JEJ, Wong JJ: Macrophage development and activation involve coordinated intron retention in key inflammatory regulators. Nucleic Acids Res. 2020;48(12):6513-6529. DOI: 10.1093/nar/gkaa435

[56] Edwards CR, Ritchie W, Wong JJ, Schmitz U, Middleton R, An X, Mohandas N, Rasko JE, Blobel GA: A dynamic intron retention program in the mammalian megakaryocyte and erythrocyte lineages. Blood.

2016;127(17):e24-e34. DOI: 10.1182/blood-2016-01-692764

[57] Llorian, M, Gooding C, Bellora N, Hallegger M, Buckroyd A, Wang X, Rajgor D, Kayikci M, Feltham J, Ule J, Eyras E, Smith CWJ: The alternative splicing program of differentiated smooth muscle cells involves concerted non-productive splicing of post-transcriptional regulators. Nucleic Acids Research. 2016;44(18):8933-8950. DOI: https://doi.org/10.1093/nar/gkw560

[58] Mauger O, Lemoine F, Scheiffele P: Targeted Intron Retention and Excision for Rapid Gene Regulation in Response to Neuronal Activity. Neuron. 2016;92(6):1266-1278. DOI: 10.1016/j.neuron.2016.11.032

[59] Naro C, Jolly A, Di Persio S, Bielli P, Setterblad N, Alberdi AJ, Vicini E, Geremia R, De la Grange P, Sette C: An Orchestrated Intron Retention Program in Meiosis Controls Timely Usage of Transcripts during Germ Cell Differentiation. Dev Cell. 2017;41(1):82-93.e4. DOI: 10.1016/j.devcel.2017.03.003

[60] Ni T, Yang W, Han M, Zhang Y, Shen T, Nie H, Zhou Z, Dai Y, Yang Y, Liu P, Cui K, Zeng Z, Tian Y, Zhou B, Wei G, Zhao K, Peng W, Zhu J: Global intron retention mediated gene regulation during CD4+ T cell activation. Nucleic Acids Res. 2016;44(14):6817-6829. DOI:10.1093/nar/gkw591

[61] Pimentel H, Parra M, Gee SL, Mohandas N, Pachter L, Conboy JG: A dynamic intron retention program enriched in RNA processing genes regulates gene expression during terminal erythropoiesis. Nucleic Acids Res. 2016;44(2):838-851. DOI: 10.1093/nar/gkv1168

[62] Wong JJ, Ritchie W, Ebner OA, Selbach M, Wong JW, Huang Y, Gao D, Pinello N, Gonzalez M, Baidya K, Thoeng A, Khoo TL, Bailey CG, Holst J, Rasko JE: Orchestrated intron retention regulates normal granulocyte differentiation. Cell. 2013 Aug 1;154(3):583-595. DOI: 10.1016/j.cell.2013.06.052

[63] Yap K, Lim ZQ, Khandelia P, Friedman B, Makeyev EV: Coordinated regulation of neuronal mRNA steady-state levels through developmentally controlled intron retention. Genes Dev. 2012;26(11):1209-1223. DOI:10.1101/gad.188037.112

[64] Rooney PH, Murray GI, Stevenson DA, Haites NE, Cassidy J, McLeod HL. Comparative genomic hybridization and chromosomal instability in solid tumours. Br J Cancer. 1999;80(5-6):862-873. DOI: 10.1038/sj.bjc.6690433

[65] Du R, Shen W, Liu Y, Gao W, Zhou W, Li J, Zhao S, Chen C, Chen Y, Liu Y, Sun P, Xiang R, Shi Y, Luo Y: TGIF2 promotes the progression of lung adenocarcinoma by bridging EGFR/RAS/ERK signaling to cancer cell stemness. Signal Transduct Target Ther. 2019;4:60. DOI: 10.1038/s41392-019-0098-x

[66] Ayee R, Ofori MEO, Wright E, Quaye O: Epstein Barr Virus Associated Lymphomas and Epithelia Cancers in Humans. J Cancer. 2020;11(7):1737-1750. DOI: 10.7150/jca.37282

[67] EBNA3 - Epstein-Barr nuclear antigen 3 - Epstein-Barr virus (strain B95-8) (HHV-4) - EBNA3 gene & protein. UniProt. Available from https://www.uniprot.org/uniprot/P12977 [Accessed:2021-01-14]

[68] Porrata-Doria, Matta T, Acevedo J: Apolipoprotein E Allelic Frequency Altered in Women with Early-onset Breast Cancer. Breast cancer : basic and clinical research.2010; 4: 43-48. DOI: 10.1177/117822341000400005.

[69] Huang Y, Mahley RW. Apolipoprotein E: structure and function in lipid metabolism, neurobiology, and Alzheimer's diseases. Neurobiology of disease. 2014; 72: 3-12. DOI: https://doi.org/10.1016/j.nbd.2014.08.025

[70] Lung Cancer Foundation of America. (2019, October 18). EGFR Mutation and Lung Cancer: What is it and how is it treated? Available from https://lcfamerica.org/lung-cancer-info/types-lung-cancer/egfr-mutation/. [Accessed: 2021-01-21]

[71] GeneCards -The Human Gene Database. EGFR gene (Protein Coding)-Epidermal growth factor receptor [Internet] . Available from https://www.genecards.org/cgi-bin/carddisp.pl?gene=EGFR. [Accessed: 2021-01-21]

[72] Lin JJ, Shaw AT: Recent Advances in Targeting ROS1 in Lung Cancer. J Thorac Oncol. 2017;12(11):1611-1625. DOI:10.1016/j.jtho.2017.08.002

[73] UniProt. ROS1 gene-Proto-oncogene Tyrosine Kinase receptor [Internet]. Available from https://www.uniprot.org/uniprot/P08922 . [Accessed: 2021-01-21]

[74] Bellissimo DC, Chen CH, Zhu Q, Bagga S, Lee CT, He B, Wertheim GB, Jordan M, Tan K, Worthen GS, Gilliland DG, Speck NA: Runx1 negatively regulates inflammatory cytokine production by neutrophils in response to Toll-like receptor signaling. Blood Adv. 2020;4(6):1145-1158. DOI: 10.1182/bloodadvances.2019000785

[75] GeneCards-The Human Gene Database. RUX1 gene (Protein Coding)-RUNX family transcription factor 1 [Internet]. Available from https://www.uniprot.org/uniprot/P08922 . [Accessed: 2021-01-20]

[76] Petitjean A, Achatz M, Borresen-Dale A, Hainaut P, Olivier M. TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene. 2007; 26:2157-2165. DOI: https://doi.org/10.1038/sj.onc.1210302

[77] MedlinePlus-Trusted Health Information. TP53 gene-tumor protein 53 [Internet]. Available from https://medlineplus.gov/genetics/gene/tp53/ . [Accessed: 2021-01-20]

[78] Sakabe NJ, de Souza SJ: Sequence features responsible for intron retention in human. BMC Genomics. 2007;8:59. DOI: 10.1186/1471-2164-8-59

[79] Melhuish TA, Wotton D: The Tgif2 gene contains a retained intron within the coding sequence. BMC Mol Biol. 2006;7:2. DOI: 10.1186/1471-2199-7-2

[80] Imotol, Pimkhaokham A, Watanabe T, Saito-Ohara F, Soeda E, Inazawa J: Amplification and overexpression of TGIF2, a novel homeobox gene of the TALE superclass, in ovarian cancer cell lines. Biochem Biophys Res Commun. 2000;276(1):264-270. DOI: 10.1006/bbrc.2000.3449

[81] Kienzle N, Young DB, Liaskou D, Buck M, Greco S, Sculley TB. Intron retention may regulate expression of Epstein-Barr virus nuclear antigen 3 family genes. J Virol. 1999;73(2):1195-1204.DOI:10.1128/JVI.73.2.1195-1204.1999

[82] Bhattacharjee S, Ghosh Roy S, Bose P, Saha A: Role of EBNA-3 Family Proteins in EBV Associated B-cell Lymphomagenesis. Front Microbiol. 2016 ;7:457. DOI:10.3389/fmicb.2016.00457

[83] Lymphoma Action.Causes of lymphoma. [Internet]. Available From: https://lymphoma-action.org.uk/about-lymphoma-what-lymphoma/causes-lymphoma [Accessed:2021-01-13]

[84] Roe CM, Fitzpatrick AL, Xiong C, Sieh W, Kuller L, Miller JP,

Williams MM, Kopan R, Behrens MI, Morris JC: Cancer linked to Alzheimer disease but not vascular dementia. Neurology. 2009;74(2):106-112. DOI: https://doi.org/10.1212/wnl.0b013e3181c91873

[85] Tzioras M, Davies C, Newman A, Jackson R, Spires-Jones T: Invited Review: APOE at the interface of inflammation, neurodegeneration and pathological protein spread in Alzheimer's disease. Neuropathol Appl Neurobiol. 2019;45(4):327-346. DOI : 10.1111/nan.12529

[86] Kloske CM, Wilcock DM. The Important Interface Between Apolipoprotein E and Neuroinflammation in Alzheimer's Disease. Front Immunol. 2020;11:754. DOI:10.3389/fimmu.2020.00754

[87] Shi Y, Yamada K, Liddelow SA, Smith ST, Zhao L, Luo W, Tsai RM, Spina S, Grinberg LT, Rojas JC, Gallardo G, Wang K, Roh J, Robinson G, Finn MB, Jiang H, Sullivan PM, Baufeld C, Wood MW, Sutphen C, McCue L, Xiong C, Del-Aguila JL, Morris JC, Cruchaga C: Alzheimer's Disease Neuroimaging Initiative, Fagan AM, Miller BL, Boxer AL, Seeley WW, Butovsky O, Barres BA, Paul SM, Holtzman DM. ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. Nature. 2017 ;549(7673):523-527. DOI: 10.1038/nature24016

[88] Liestøl K, Kvittingen EA, Rootwelt H, Dunlop O, Goplen AK, Pedersen JC, Brorson SH, Børresen-Dale AL, Myrvang B, Maehlen J: Association between apolipoprotein E genotypes and cancer risk in patients with acquired immunodeficiency syndrome. Cancer Detect Prev. 2000;24(5):496-499.

[89] Zheng JT, Lin CX, Fang ZY, Li HD. Intron Retention as a Mode for RNA-Seq Data Analysis. Frontiers in Genetics. 2020;11:1-7. DOI: https://doi.org/10.3389/fgene.2020.00586

[90] Zhang Q, Li H, Jin H, Tan H, Zhang J, Sheng S. The global landscape of intron retentions in lung adenocarcinoma. BMC Med. Genomics 2014;7:15. DOI: 10.1186/1755-8794-7-15

[91] Vallath S, Hynds RE, Succony L, Janes SM, Giangreco A: Targeting EGFR signalling in chronic lung disease: therapeutic challenges and opportunities. Eur Respir J. 2014;44(2):513-522. DOI:10.1183/09031936.00146413

[92] Sood R, Kamikubo Y, Liu P: Role of RUNX1 in hematological malignancies. Blood. 2017;129(15):2070-2082. DOI: 10.1182/blood-2016-10-687830

[93] Cooks T, Harris CC: p53 mutations and inflammation-associated cancer are linked through TNF signaling. Mol Cell. 2014;56(5):611-612. DOI: 10.1016/j.molcel.2014.11.018

[94] Uehara I, Tanaka N: Role of p53 in the Regulation of the Inflammatory Tumor Microenvironment and Tumor Suppression. Cancers (Basel). 2018;10(7):219. DOI:10.3390/cancers10070219

[95] Wang MC, Li CL, Cui J, Jiao M, Wu T, Jing LI, Nan K J. BMI-1, a promising therapeutic target for human cancer. Oncology letters. 2015; 10(2): 583-588. DOI: https://doi.org/10.3892/ol.2015.3361

[96] GeneCards-The Human Gene Database. BMI 1 Gene (Protein Coding),BMI 1 Protooncogene, Polycomb Ring Finger [Internet]. Available from https://www.genecards.org/cgi-bin/carddisp.pl?gene=BMI1 . [Accessed: 2021-01-20]

[97] Pallante P, Forzati F, Federico A, Arra C, Fusco A. Polycomb protein

family member CBX7 plays a critical role in cancer progression. American journal of cancer research. 2005; 5(5): 1594-1601.

[98] GeneCards-The Human Gene Database. CBX7 Gene (Protein Coding) Chromobox 7 [Internet]. Available from https://www.genecards.org/cgi-bin/carddisp.pl?gene=CBX7 . [Accessed: 2021-01-20]

[99] Tyagi A, Sarodaya N, Kaushal K, Chandrasekaran AP, Antao AM, Suresh B, Rhie BH, Kim KS, Ramakrishna S. E3 Ubiquitin Ligase APC/CCdh1 Regulation of Phenylalanine Hydroxylase Stability and Function. International Journal of Molecular Sciences. 2020; 21(23):9076. DOI: https://doi.org/10.3390/ijms21239076

[100] MedlinePlus-Trusted Health Information. PAH gene-phenylalaline hydroxylase [Internet]. Available from https://medlineplus.gov/genetics/gene/pah/ [Accessed: 2021-01-20]

[101] Shen J, Li P, Shao X, Yang Y, Liu X, Feng M, Yu Q, Hu R, Wang Z. The E3 Ligase RING1 Targets p53 for Degradation and Promotes Cancer Cell Proliferation and Survival. Cancer Research. 2017; 78(2): 359-371. DOI: https://doi.org/10.1158/0008-5472.can-17-1805

[102] GeneCards-The Human Gene Database. RING 1(Protein Coding) -RING Finger Protein 1 [Internet]. Available from https://www.genecards.org/cgi-bin/carddisp.pl?gene=RING1 . [Accessed:2021-01-20]

[103] Lee JY, Kong G. MEL-18, a tumor suppressor for aggressive breast cancer. Oncotarget.2015;6(18):15710-15711.

[104] GeneCards-The Human Gene Database. PCGF2 Gene(Protein Coding), Polycomb group ring finger 2 [Internet]. Available from https://www.genecards.org/cgi-bin/carddisp.pl?gene=PCGF2 [Accessed :2021-01-22]

[105] Crea F, Paolicchi E, Marquez VE, Danesi R. Polycomb genes and cancer: Time for clinical application? Critical Reviews in Oncology/Hematology. 2012; 83(2): 184-193. DOI: https://doi.org/10.1016/j.critrevonc.2011.10.007

[106] MedlinePlus-Trusted Health Information. EZH2 gene-enhancher of zeste 2 polycomb repressive complex 2 subunit [Internet]. Available from https://medlineplus.gov/genetics/gene/ezh2/ [Accessed : 2021-01-19]

[107] Seo GS, Yu JI, Chae SC, Park WC, Shin SR, Yoo ST, Choi SC, Lee SH: (2013). EED gene polymorphism in patients with colorectal cancer. The International journal of biological markers. 2013; 28(3): 274-279. DOI: https://doi.org/10.5301/JBM.5000024

[108] Yu W, Du H. Roles of Polycomb gene EED in pathogenesis and prognosis of acute myeloid leukemia and diffuse large B cell lymphoma. 2018;1-21. DOI: https://doi.org/10.1101/444745

[109] GeneCards-The Human Gene Database. EED gene-Embryonic Ectoderm Development [Internet] Available from https://www.genecards.org/cgi-bin/carddisp.pl?gene=EED [Accessed : 2021-01-21]

[110] Wu Y, Hu H, Zhang W, Li Z, Diao P, Wang D, Zhang W, Wang Y, Yang J, Cheng J. SUZ12 is a novel putative oncogene promoting tumorigenesis in head and neck squamous cell carcinoma. Journal of cellular and molecular medicine. 2018; 22(7): 3582-3594. DOI: https://doi.org/10.1111/jcmm.13638

[111] GeneCards-The Human Gene Database. SUZ12 Gene(Protein Coding), SUZ12 Polycomb Repressive Complex 2 subunit [Internet]. Available from https://www.genecards.org/cgi-bin/carddisp.pl?gene=SUZ12 . [Accessed: 2021-01-20]

[112] Jain P, Ballare C, Blanco E, Vizan P, Di Croce L. PHF19 mediated regulation of proliferation and invasiveness in prostate cancer cells. ELife. 2020; 9: 1-11. DOI: https://doi.org/10.7554/elife.51373

[113] GeneCards-The Human Gene Database. PHF19 Gene(Protein Coding), PHD Finger Protein 19 [Internet]. Available from https://www.genecards.org/cgi-bin/carddisp.pl?gene=PHF19 [Accessed: 2021-01-20]

[114] National Cancer Institute. NCI Dictionary of Cancer Terms [Internet]. Available from https://www.cancer.gov/publications/dictionaries/cancer-terms/def/kras-gene [Accessed: 2021-01-20]

[115] MedlinePlus Genetics. KRAS gene: MedlinePlus Genetics [Internet]. 2020, August 18. Available from https://medlineplus.gov/genetics/gene/kras/ [Accessed: 2021-01-19]

[116] Vidyasagar, A. What Is CRISPR? [Internet]. 2018, April 21. Available from https://www.livescience.com/58790-crispr-explained.html. [Accessed: 2021-01-19]

[117] Heler R, Marraffifini LA, Bikard D. Adapting to new threats: the generation of memory by CRISPR Cas immune systems. Mol. Microbiol. 2014; 93(1):1-9. DOI: 10.1111/mmi.12640

[118] Marraffifini LA. CRISPR-Cas immunity in prokaryotes. Nature . 2015; 526(7571):55-61. DOI: 10.1038/nature15386

[119] Mojica FJM, Rodriguez-Valera F. The discovery of CRISPR in archaea and bacteria. FEBS J. 2016; 283(17):3162-3169. DOI: 10.1111/febs.13766

[120] Jiang F, Doudna JA. CRISPR–Cas9 Structures and Mechanisms. Annual Review of Biophysics. 2017; 46(1): 505-529. DOI: https://doi.org/10.1146/annurev-biophys-062215-010822

[121] Mohamadi S, Bostanabad SZ, Mirnejad R. CRISPR Arrays: A Review on Its Mechanism. Journal of Applied Biotechnology Reports. 2020; 7(2): 81-86. DOI: 10.30491/jabr.2020.109380