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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

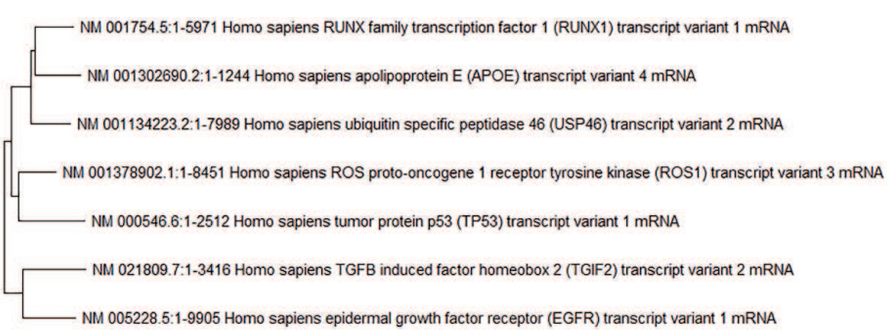

Figure 1.
Phylogenetic tree of intron retaining (IR) genes.

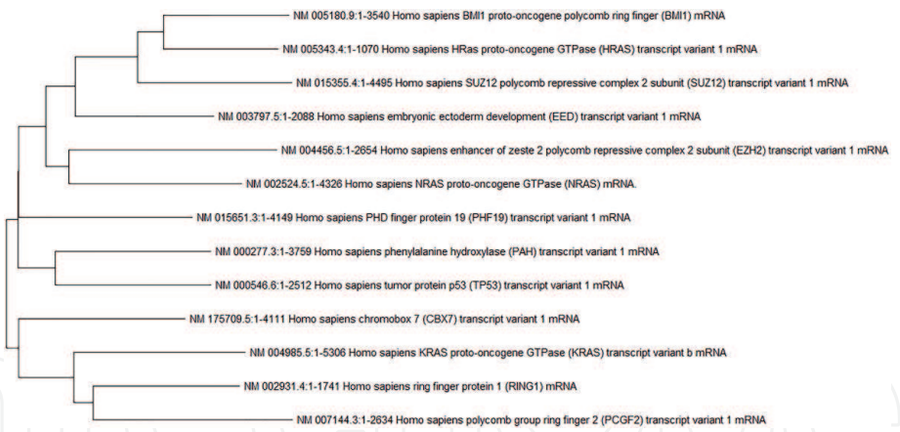

Figure 2.
Phylogenic tree between RAS oncogenes and PcGs.

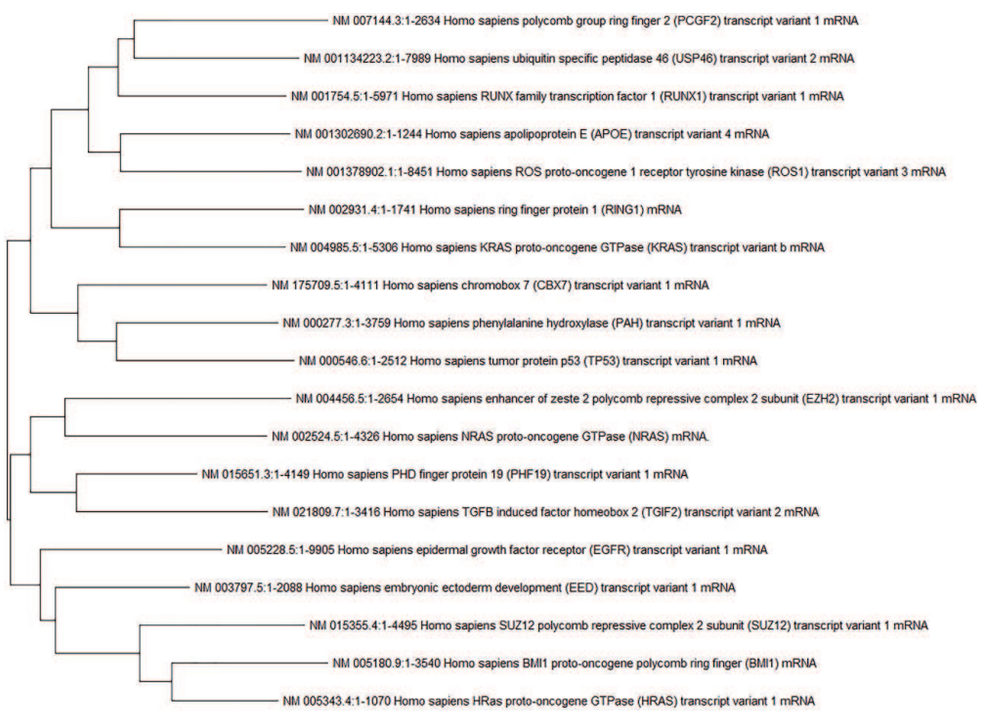

Figure 3.
Phylogenic tree among IR gene, RAS oncogenes and PcGs.

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

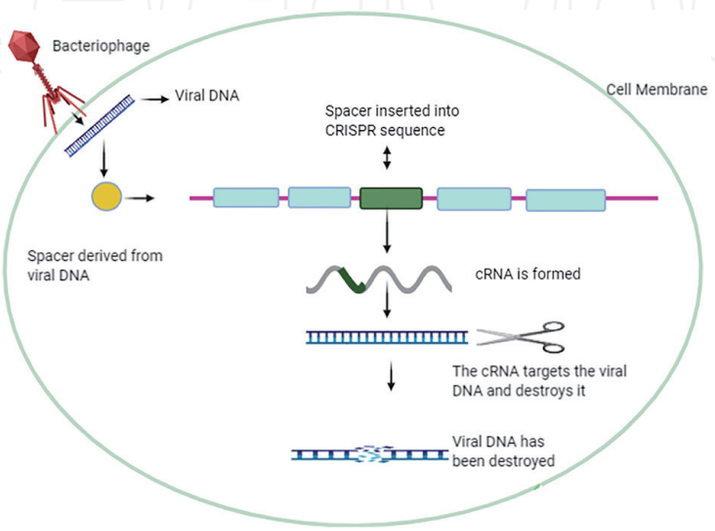

Figure 4.
CRISPR biology.

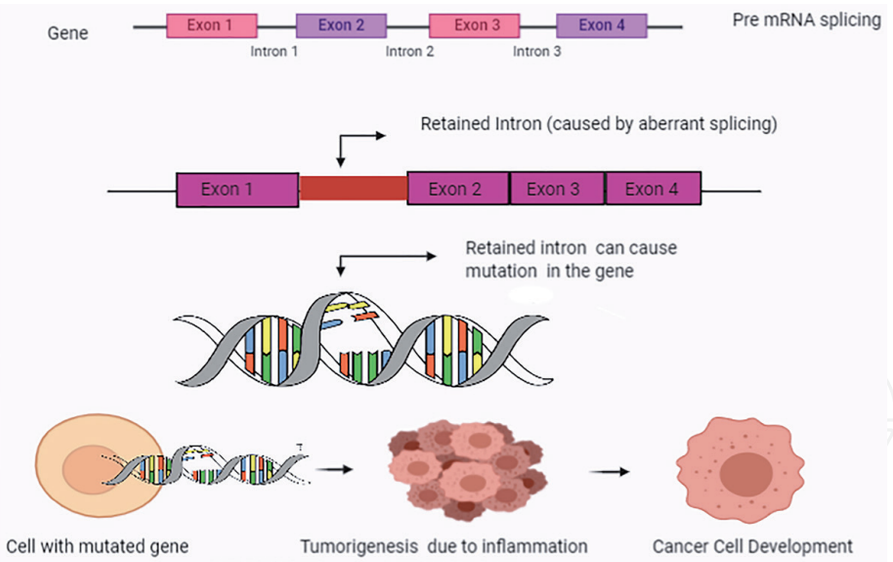

Figure 5.
Illustration of retained intron in the gene causing mutation which leads to inflammation and tumorigenesis resulting in development of cancer.

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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