Acta Pharmaceutica Sinica B 2024;14(11):4637–4648

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

An overview of the functions and mechanisms of APOBEC3A in tumorigenesis

Yuqi Yang [a,b,†], **Nan Liu** [a,b,†], **Likun Gong** [a,b,*]

[a]*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*
[b]*University of Chinese Academy of Sciences, Beijing 100049, China*

Received 23 March 2024; received in revised form 6 June 2024; accepted 26 July 2024

KEY WORDS

APOBEC3A;
Deaminase;
Mutation;
DNA damage;
Tumorigenesis;
Tumor-associated viruses;
Macrophage;
Tumor microenvironment

Abstract The APOBEC3 (A3) family plays a pivotal role in the immune system by performing DNA/RNA single-strand deamination. Cancers mostly arise from the accumulation of chronic mutations in somatic cells, and recent research has highlighted the A3 family as a major contributor to tumor-associated mutations, with A3A being a key driver gene leading to cancer-related mutations. A3A helps to defend the host against virus-induced tumors by editing the genome of cancer-associated viruses that invade the host. However, when it is abnormally expressed, it leads to persistent, chronic mutations in the genome, thereby fueling tumorigenesis. Notably, A3A is prominently expressed in innate immune cells, particularly macrophages, thereby affecting the functional state of tumor-infiltrating immune cells and tumor growth. Furthermore, the expression of A3A in tumor cells may directly affect their proliferation and migration. A growing body of research has unveiled that A3A is closely related to various cancers, which signifies the potential significance of A3A in cancer therapy. This paper mainly classifies and summarizes the evidence of the relationship between A3A and tumorigenesis based on the potential mechanisms, aiming to provide valuable references for further research on the functions of A3A and its development in the area of cancer therapy.

*Corresponding author.
E-mail address: lkgong@cdser.simm.ac.cn (Likun Gong).
†These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2024.08.020

1. Introduction

The apolipoprotein B mRNA-editing catalytic polypeptide-like 3 (APOBEC3, A3) enzyme family consists of seven deaminase members that play a pivotal role in the innate immunity defense against viral infections. In addition to extending the biological roles of the A3 family, the new revelation that they may also cause mutations in cellular genomic DNA suggests a possible connection between the A3 family and the chronic mutations occurring in human cancers[1,2]. The Cancer Genome Atlas database has further corroborated the presence of APOBEC-characterized mutations in multiple tumors[3]. These chronic mutations have been identified in roughly 15% of the sequenced human tumors, with A3A, A3B, and A3H Haplotype I (Hap I) being the principal genes involved[4-6]. While prior studies on the A3 family primarily focused on their biological role in antiviral innate immunity, further work is still needed to unravel how these enzymes exert their "off-target" effect on genomic DNA and to delineate the precise mechanisms underlying these effects.

Studies have shown that A3A stands as the primary driver gene for cancer-associated mutations within the A3 family[7-10]. The distinctive mutation pattern of A3A has been identified in a wide range of cancers[11,12], triggering damage responses in the cellular genome[13,14], thereby fueling tumorigenesis. A growing number of studies have been devoted to unraveling the pivotal role of A3A in tumorigenesis[7,8,15]; however, studies targeting different tumor types have yielded inconsistent conclusions, reflecting differences in the underlying pathways. Conversely, the genome editing effect of A3A on tumor cells may hinder tumor growth by causing genomic instability or the production of neoantigens[16,17], which help immune cells identify and eradicate tumors. Elucidating the role of A3A in different tumors is critical for continued developmental research into this target.

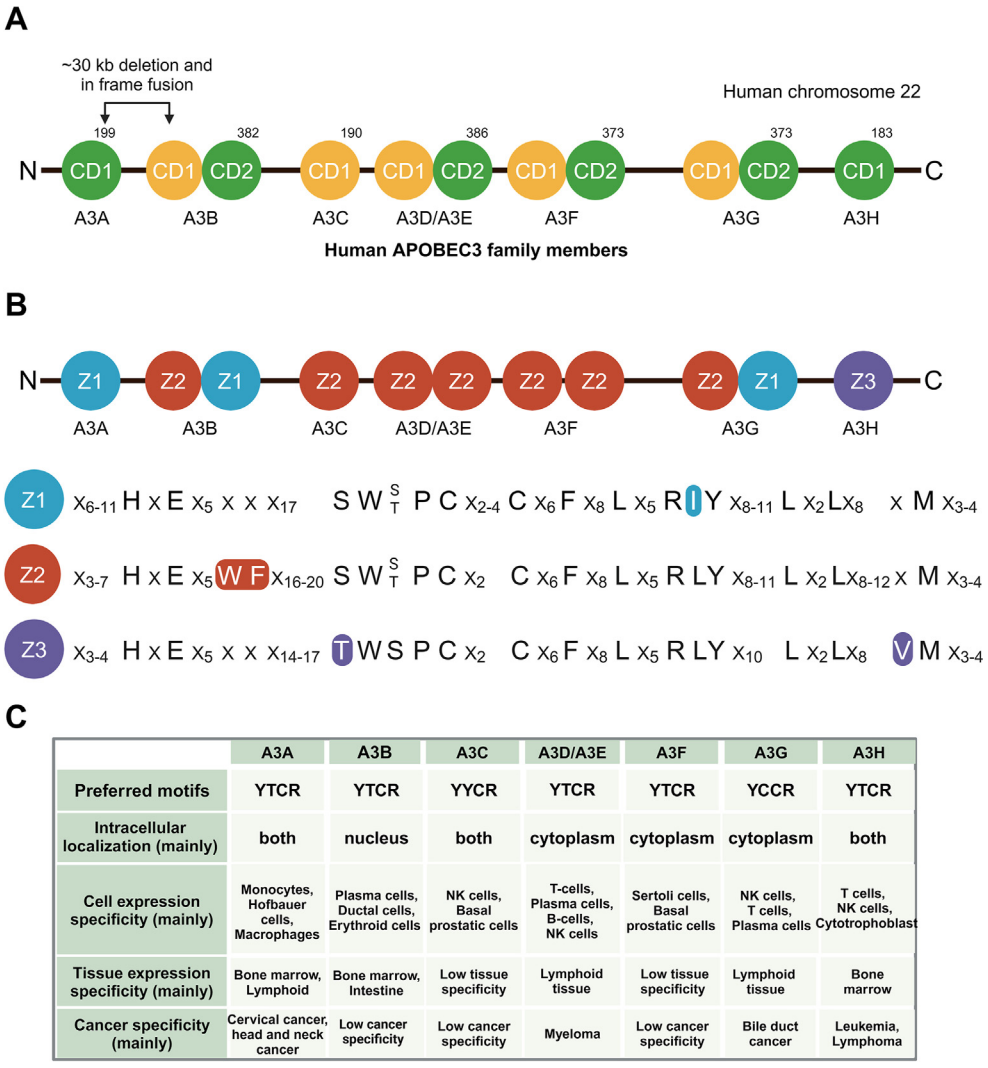
Notably, A3A is highly expressed in immune cells, especially macrophages. Research has confirmed that A3A can influence the polarization state of macrophages[18,19], thereby enhancing the tumor-inhibitory capabilities of tumor-associated macrophages in the tumor microenvironment. Additionally, studies have confirmed that A3A expression in T cells can also impact the function of T cells[20]. A comprehensive synthesis of these diverse studies could provide novel insights into the pathways associated with A3A, thereby accelerating its potential application as a tumor therapeutic target. This review first summarizes the cancer types and research progress related to A3A expression in existing studies, provides a comprehensive overview and classification of the proposed mechanisms of A3A action, and points out problems and possible research ideas based on these clinical and *in vitro* or *in vivo* mechanism explorations.

2. The function of A3A and its association with tumorigenesis

The seven members of the A3 family are arranged in tandem on human chromosome 22, each containing one or two cytosine deaminase (CD) domains[21]. A3A, A3C and A3H contain a single CD domain. In contrast, A3B, A3D, A3F, and A3G contain two CD domains, of which only CD2 located at the C-terminus is catalytically active, while CD1 mediates functions such as RNA binding and viral envelopment[22,23] (Fig. 1). According to the specificity of amino acids, there are three types of CD domains sharing a consensus Zn-binding motif His−X23−28−Pro−Cys−X2−4−Cys (X can be any amino acid), named Z1, Z2, and Z3, respectively[24]. The specificity and conservation of the CD domains may play a role in determining the functional similarities and differences among the members of the A3 family. One crucial function of A3 proteins within the innate immune system is to inhibit foreign retroviruses. Among these proteins, A3G was the first member identified to possess a notable antiretroviral effect[25,26]. A3A also exhibits potent inhibition of endogenous retrotransposons, including long or non-long terminal repeat-retrotransposons[27-29], and a wide range of other viruses, playing a crucial role in the human innate immune response against pathogens[28,30]. However, in contrast to A3G, A3A has no effect on human immunodeficiency virus (HIV) infection in multiple cell lines[31], possibly related to the distinct mechanisms of retroviral suppression by A3G and A3A. A3G primarily functions as a CD during reverse transcription after being packaged into virus, while A3A directly prevents viral entry into the target cell during the early stages of the infection in a deaminase-dependent or non-dependent manner[32-34]. Even though A3A does not target HIV, when the structural domain of A3G that specifically targets HIV is fused to the A3A protein, the resulting fusion protein can induce editing of the viral genome and block replication of the retrovirus once inside it has entered retroviral capsid[35]. A3A can also edit cancer-associated viral genomes[36] and induce genomic mutations. Studies showed that A3A could induce high-frequency C−U mutations in the papillomavirus genome as well as in transfected plasmid DNA[37,38]. It is important to note that A3A exclusively acts on single-strand DNA (ssDNA), thus, the above effects on papillomavirus and plasmid DNA may only occur during the transient single-stranded phase.

The expression and functions of A3A are regulated by a variety of factors. It has been observed that interferon IFN-α or IFN-γ could stimulate the expression of A3A[37], and that toll-like receptor signaling can similarly up-regulate A3A expression in hematopoietic cells *via* CpG-DNA. Besides these factors, changes in pH within the organism also affect the specificity and deaminase activity of A3A[39]. Under strict YYCR (Y for pyrimidine; R for purine) conditions, A3A displays optimal CD activity at pH 5.6−6.1, whereas at pH 7.4−7.8 the CD activity reduces by 13−30-fold and specificity is also decreased. Unlike other members of the A3 family, overexpression of A3A in the 293 or Hela cell lines has no significant inhibitory effect on retroviruses[40,41]. However, recent studies have unveiled a unique role for A3A in this family. It localizes exclusively in myeloid cells of HIV-1-targeted leukocytes, and its expression level specifically spikes as HIV-1 spreads in infected primary macrophages[33]. The researchers silenced A3A in THP-1, primary macrophages, and dendritic cells, which markedly increased their susceptibility to HIV-1 infection. Conversely, silencing A3A in primary blood cells (depleted of monocytes) or ectopic expression of A3A in other cell lines had negligible effects. These findings suggest that A3A exerts anti-lentiviral activity selectively in myeloid-derived cells and only in cells in which it is naturally highly expressed.

Some members of the A3 family are localized and active in the nucleus, yet the potential roles of these proteins in affecting cellular genomic DNA remain to be explored. In 2012, Nik-Zainal et al.[42] analyzed somatic mutations in 21 different breast cancers and revealed a localized hypermutation phenomenon known as "kataegis". This discovery marked the first proposed link between the APOBEC cytosine enzyme family and tumorigenesis. Different from many other mutations confined to specific cancer types, APOBEC cytosine family-associated mutations occur in diverse cancer categories. To date, the APOBEC-associated mutational signature has been validated in more than 70% of



C

	A3A	A3B	A3C	A3D/A3E	A3F	A3G	A3H
Preferred motifs	YTCR	YTCR	YYCR	YTCR	YTCR	YCCR	YTCR
Intracellular localization (mainly)	both	nucleus	both	cytoplasm	cytoplasm	cytoplasm	both
Cell expression specificity (mainly)	Monocytes, Hofbauer cells, Macrophages	Plasma cells, Ductal cells, Erythroid cells	NK cells, Basal prostatic cells	T-cells, Plasma cells, B-cells, NK cells	Sertoli cells, Basal prostatic cells	NK cells, T cells, Plasma cells	T cells, NK cells, Cytotrophoblast
Tissue expression specificity (mainly)	Bone marrow, Lymphoid	Bone marrow, Intestine	Low tissue specificity	Lymphoid tissue	Low tissue specificity	Lymphoid tissue	Bone marrow
Cancer specificity (mainly)	Cervical cancer, head and neck cancer	Low cancer specificity	Low cancer specificity	Myeloma	Low cancer specificity	Bile duct cancer	Leukemia, Lymphoma

Figure 1 Arrangement of A3 family members on chromosomes and expression profile. (A) The seven members of the A3 family are arranged in tandem on human chromosome 22. The CD domain is shown, yellow indicates deaminase inactive, green indicates active, and the number represents the number of amino acids. (B) A3 family proteins were displayed according to their Zn-binding motifs. The catalytic domains Z1, Z2, and Z3 are colored in blue, red, and purple, respectively. The amino acid sequences of each Z-motif are shown. Group-specific distinctions of Z-domains are highlighted, and an "x" specifies nearly any amino acid. (C) Preferred motifs, intracellular localization, cell or tissue expression specificity, and cancer specificity of A3 family members. Y for pyrimidine; R for purine. The above data comes from The Human Protein Atlas database. All figures in this review article were created by BioRender.com.

tumor types and approximately 50% of oncogenes, including breast cancer, papilloma, and others[3,43,44]. Subsequent studies have consistently demonstrated that A3 family-associated mutations are the second most frequently detected mutational signature in human cancer tissues, after those associated with aging[3,8,45]. Genomic mutations associated with the A3 family are typically characterized by cytosine deamination-like single base substitutions (SBSs). These mutations can be categorized as non-clustered (SBS2 and SBS13) and clustered (kataegis and omikli). Instead of being randomly distributed[42,46], they are enriched in stem-loop structure in the form of YTCA characteristic mutant sequences (C-mutant bases)[4]. The involvement of the A3 family in genomic DNA editing may contribute to genomic instability, which could trigger cancer. While all members of this family may play a role in this process, numerous studies have highlighted the pivotal role of A3A[8,15,47]. The presence of A3A proteins in both the cytoplasm and the nucleus indicates that they are involved in gene mutations not only in the nucleus but also in mitochondrial DNA[21,48]. A3A causes DNA strand breaks in somatic cells in a deaminase activity-dependent manner, which in turn triggers the DNA damage response (DDR), activates DNA replication checkpoints, and induces cell cycle arrest, leading to the formation of tumor-associated mutations. A study utilizing the murine Fah liver complementation and regeneration system demonstrated that expression of A3A alone, without concomitant inhibition of TP53, drives hepatocellular carcinoma in mice and that this effect is dependent on the DNA deamination activity of A3A[49]. Researchers constructed mouse models of colorectal and hepatocellular carcinoma using A3A-expressing mice and found that A3A expression promoted tumorigenesis, a function that has not been extensively documented in any other A3 family member[7]. Furthermore, analysis of APOBEC-characteristic mutations in

Table 1 Summary of A3A-related tumor types and research progress.

Cancer type	Research type	Sample type	Role of A3A	Method	Ref.
Bladder cancer	Clinical	/	/	/	
	Non-clinical	Tumor cells	Main driver of mutations	Genomics analyses	50
Breast cancer	Clinical	138 tumor and adjacent tissues	Increased risk	qPCR	51
	Non-clinical	Tumor cells	Drives carcinogenesis	Genomics analyses	
Cervical cancer	Clinical	91 tumor tissues	Connect with good prognosis	IHC	52
	Non-clinical	Tumor cells	Induces apoptosis	Genomics analyses	16
Colon and rectal cancer	Clinical	/	/	/	
	Non-clinical	Mouse models	Promotes tumor formation	Transgenic mouse	7
Liver cancer	Clinical	/	/	/	
	Non-clinical	Mouse models	Promotes tumor formation	Transgenic mouse	7
Lung cancer	Clinical	62 tumor tissues	Promotes drug resistance	Genomics analyses	
	Non-clinical	Tumor cells	Promotes drug resistance	Genomics analyses	15
Lymphoma cancer	Clinical	/	/	/	
	Non-clinical	Tumor cells	Main driver of mutations	Genomics analyses	50
Melanoma	Clinical	/	/	/	
	Non-clinical	Mouse models	Tumor suppression	Intratumoral electroporation	53
Oropharyngeal cancers	Clinical	65 tumor tissues	Integration of human papillomavirus (HPV) DNA	3D-PCR and DNA-seq	
	Non-clinical	/	/	/	54
Ovarian cancer	Clinical	380 tumor tissues	Biomarker for immunotherapy	RNA-seq	55
	Non-clinical	/	/	/	
Pancreatic cancer	Clinical	93 tumor and adjacent tissues	Homozygous deleted in tumors	Genomics analyses	56
	Non-clinical	Tumor cells	Promotes cancer metastasis	Genomics analyses	57
Penile squamous cell Carcinoma	Clinical	34 tumor tissues	Inhibit invasiveness	IHC	58
	Non-clinical	/	/	/	

mouse tumor DNA supported a causal relationship between A3A-catalyzed deamidation and tumor-induced mutation generation[7]. The research progress of A3A in a variety of tumors is shown in Table 1. In addition to genetic mutations, numerous studies have established a strong association between inflammation and cancer. Given the contribution of A3A to the immune response and the role of nucleic acid deaminase, it is conceivable that A3A plays a pivotal role in tumorigenesis. Overall, although A3A plays an important role as a cellular defense protein, its DNA-mutagenic activity may also be detrimental to A3A-expressing cells.

3. Role and mechanism of A3A in tumorigenesis

3.1. A3A's function in editing the genome of tumor-associated viruses within target cells

Approximately 7% of human cancers are caused by viral infections, including HPV, hepatitis B and C viruses (HBV and HCV), etc.[59]. While these viruses do encode potent oncoproteins that target cellular tumor suppressors, their presence is usually not sufficient to cause cancer, which is a multifactorial process involving multiple mechanisms. For instance, most people will be exposed to HPV at some point in their lives, but in most cases, the immune system will naturally eradicate the virus. Hyper-editing of the HPV genome was initially observed in HPV-positive papilloma cancer patients. Subsequent studies confirmed that these edits exhibited the A3 deaminase signature. This finding was validated by 3D-PCR and *in vitro* experiments of co-transfection of HPV plasmids with A3 enzymes, including A3A, A3C, and A3H[38]. Additionally, researchers observed that HPV16 sequences were edited with a unique A3 family signature in precancerous cervical biopsies, while A3B and A3A expression levels were markedly elevated in HPV-positive keratinocytes and cervical tissues during the early stages of cancer progression[60]. These findings collectively suggest that sporadic or transient overexpression of A3 proteins may mutate the viral genome, thereby affecting tumorigenesis and its progression (Fig. 2). Furthermore, other studies have indicated that although the HPV genome gradually loses A3-preferred targeting sequences over time[61], these viruses are still affected by A3A activity. Researchers speculate that the continuous editing of A3A may facilitate viral evolution and immune escape[62]. However, the veracity of this speculation remains to be conclusively determined.

A study by Chen et al.[63] found that overexpression of A3A in cervical cancer cell lines exerted a notable inhibitory effect on cell growth, migration, and invasion. Additionally, A3A promoted apoptosis of tumor cells in a deaminase-dependent manner, and its overexpression decreased the expression of key proteins associated with HPV infection, including HPV16-E6, HPV16-E7, and HPV18-E6. In another study, experiments using NIKS cells with and without the expression of HPV16 oncoprotein E7 revealed that the E7 oncoprotein can induce A3A expression *via* the dsRNA-sensing IFN−STAT2 pathway or the DDR associated NF-κB pathway[64] (Fig. 2). Remarkably, knockdown of A3A in human keratinocytes resulted in a significant increase in HPV infectivity[60]. A study has shown that the baseline expression levels of A3 family members (except for A3B) are notably elevated in normal mucosal tissues such as the cervix and vagina compared to non-cutaneous tissues[61]. This heightened A3A expression appears to play a pivotal role in enhancing resistance to viral infection, thus serving as evidence for related antitumor potential.

Similarly, in a study investigating the targeted destruction mechanism of HBV covalently closed circular DNA (cccDNA), researchers discovered that IFN-α agonists have the potential to

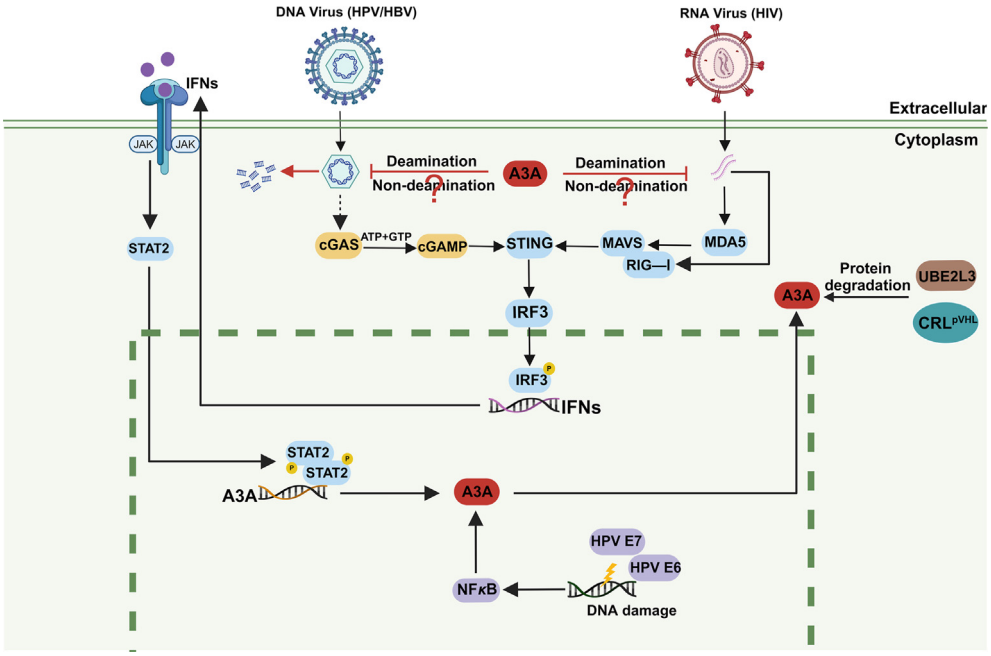


Figure 2 The underlying mechanisms of A3A editing tumor-associated viruses. Invading viruses are recognized by the DNA-sensor cGAS or the RNA-sensor MDA5, which then triggers the IFN−STAT pathway, leading to the upregulation of A3A expression. Additionally, the HPV E6/E7 protein can activate the NF-κB pathway, resulting in increased expression of A3A. Subsequently, A3A inhibits virus invasion through deaminase-dependent or independent mechanisms. Besides, A3A expression is dysregulated by protein degradation through the ubiquitin−proteasome pathway.

induce A3A expression. This induction, in turn, triggers the deamination and subsequent degradation of HBV cccDNA[65,66]. Invading HBV may elevate A3A expression through a double-stranded DNA (dsDNA)-sensing mechanism, akin to the process observed with HPV. The resulting A3A production facilitates the degradation of cccDNA into fragments (Fig. 2). However, persistently high expression of A3 proteins induced by chronic infection may be an important predisposing factor for somatic mutations in normal tissues, even prior to the development of cancer. A3 activity can be regulated by post-translational processes, particularly ubiquitin-mediated protein degradation pathways. For example, the von Hippel Lindau tumor suppressor induces the degradation of all seven A3 family members *via* its Cullin-RING E3 ubiquitin ligase[67]. High levels of ubiquitin ligase 2-UBE2L3 have also been shown to facilitate the degradation of A3A protein[68]. Of note, HBV cccDNA is stabilized by the high levels of UBE2L3 through mediating A3A protein degradation[68]. We need to evaluate the effect of A3A degradation on its antiviral and antitumor effects in subsequent investigations.

3.2. A3A induces tumorigenesis by mutating genomic DNA in a deaminase-dependent manner

Apart from their effects on foreign viruses, aberrant expression of the A3 family also causes mutations in somatic and mitochondrial DNA. These proteins are known to act specifically on ssDNA, which is commonly seen during genome DNA replication and transcription. Thus, these proteins may alter the human genomic DNA by inducing persistent mutations[69] (Fig. 3). Various A3 family members have been found to have varying degrees of nucleic acid editing ability in the host. For instance, mitochondrial DNA can be edited by five family members (A3A, A3C, A3F, A3G, and A3H), whereas nuclear DNA is primarily edited by A3A[70]. This finding emphasizes the significance of the A3 family in cancer evolution, especially A3A, whose expression increases in the context of inflammation. Moreover, the recurrent low-level mutations induced by A3A can catalyze the transformation of healthy genes into oncogenes. A3A deaminates the non-transcribed strand in an *in vitro* transcription model[39] and

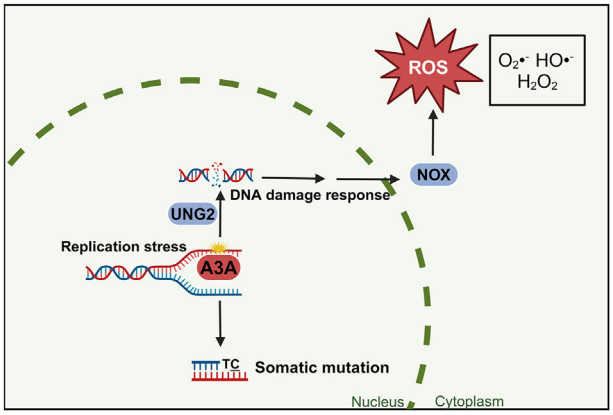


Figure 3 A3A induces genomic mutations in normal cells to promote tumorigenesis. A3A induces persistent somatic mutations in healthy cells, promoting their transformation into tumor cells. The point mutations initiated by A3A lead to DNA double-strand breaks, which are then under the influence of UNG2, triggering a DNA damage response. This response subsequently promotes the production of reactive oxygen species (ROS) through NOX enzymes, creating a tumor-promoting environment.

overexpression of A3A and A3B in yeast also induces a series of breakage-associated mutations by deamidating ssDNA[71]. All of them are similar to the "kataegis" mutations originally identified by Nik-Zainal et al.[42], which are mainly cytosine alterations, including C to T transitions and C to G transversions. The above phenomena are attributable to clustered deamination of cytosine residues occurring on ssDNA, which is produced during processes like replication or recombination repair. Subsequently, this triggers uracil excision and initiates the DDR mechanism, which leads to the formation of base-free sites and induces error-prone DNA synthesis of complementary DNA strands, such as the insertion of adenine (transition outcome) and guanine (transversion outcome)[72].

To confirm that A3A activates the DDR signaling pathway, researchers examined checkpoint signaling and cell cycle progression in stable cell lines overexpressing A3A. They found that A3A strongly activates key mediators in the DDR pathway, including phosphorylation of the histone variant H2AX at Ser139 and the recruitment of proteins such as 53BP1 and replication protein A[14,70,73-75]. When cells undergo replicative stress, their genomic DNA is more susceptible to A3A-induced damage, and proliferating immature cells are also more susceptible to A3A-induced mutations compared to non-replicating cells[70]. Given the rapidly proliferative properties of tumor cells. It is conceivable that the activity of A3A within tumor cells could be utilized as a potential avenue for tumor therapy, which is achieved by inducing genotoxicity and causing intracellular damage[76,77]. Recent studies have also shown that genomic alterations (mutation burden) in tumor cells increase the generation of tumor neoantigens, which leads to increased tumor sensitivity to immune checkpoint blockade[78-80]. However, a study of A3 mutations in lung cancer patients found that targeted therapies for lung cancer induced A3A expression and drove the evolution of drug-resistant cancer cells[15] (Fig. 4). Moreover, deletion of A3A reduced A3-associated mutations in drug-resistant cells and slowed down the development of drug resistance. Inhibition of A3A expression or activity may be a potential strategy for overcoming resistance to targeted therapies for lung cancer[15]. The above results suggest that A3A has great potential in tumor therapy, but due to its diverse roles in different tumor types, we need to explore the role of A3A in specific tumors clearly in order to promote the application of A3A in tumor therapy.

Analysis of mutant sequence data in breast cancer revealed that A3A and A3B were the only deaminases with mutation patterns consistent with those observed in patients, suggesting that A3A and A3B may be the key deaminases responsible for the hypermutation phenomena in breast cancer. Mia Petljak and colleagues[50] revealed that A3B could exert an inhibitory effect on A3A-dependent mutations. They then knocked out the A3A and A3B genes in tumor cell lines, respectively, and analyzed the naturally occurring mutations in the cell lines over time. Surprisingly, despite relatively low expression levels of A3A and no detectable changes in its editing enzyme activity, deletion of A3A resulted in a significant reduction in A3-associated mutations (SBS2 and SBS13 mutations) in the tumor cell lines. These mutations are responsible for a large portion of the A3-associated mutational signature observed in cancer. Furthermore, the deletion of A3B increased the expression, activity, and mutational effects of A3A. A3B is effectively eliminated by a frequent germline deletion in humans that consists of a 25-kilobase segment polymorphism, enabling persistent expression of the A3A-A3B hybrid transcript[81,82]. Evidence has revealed a noteworthy difference in the activity of the A3A protein derived from the A3A-UTR A3B transcript compared to that from the A3A-UTR A3A transcript, with the former exhibiting a 20-fold higher activity[82], suggesting that A3B may play a role in tumor regulation by influencing the expression of A3A. However, more research is necessary to determine how the 25-kilobase deletion in the A3 locus, which results in the A3B deletion and A3A overexpression, affects particular cancer types.

3.3. A3A affects macrophage polarization involved in tumorigenesis

Macrophages are tissue-localized myeloid cells that regulate host defense, maintain internal immune homeostasis, and serve as crucial antigen-presenting cells involved in initiating specific T-cell responses[83]. Furthermore, macrophages excel at removing cellular debris and apoptotic cells through phagocytosis, a process that profoundly influences the regulation of immune responses against viruses and tumors[84]. In addition to $CD4^+$ T cells, $CD4^+CCR5^+$ macrophages also serve as primary targets and hosts for HIV-1[85], and they exhibit higher susceptibility to infection compared to immature monocytes. It's significant to note that macrophages typically survive viral infection and continue to exist in tissues as virus hosts for extended periods of time[86]. In a study examining differences in the susceptibility of myeloid cell populations to viruses, Peng et al.[87] found that A3A was highly expressed in monocytes and tended to decline as monocytes differentiated and matured, which accounts for the relatively higher resistance of monocytes to viruses. Studies have demonstrated that modulation of A3A expression in various myeloid lineages produces diverse responses to HIV-1, which may be dependent or independent of its deaminase action[28,88], suggesting that A3A may regulate monocyte resistance to infection through pathways distinct from those of the A3G and other family members. Sharma et al.[19] revealed that macrophages and monocytes undergo site-specific C>U RNA editing on hundreds of gene transcripts during M1 polarization in response to stimuli such as hypoxia and interferon and that this process is dependent on the deaminase activity of A3A. Aberrant RNA editing alters the amino acid sequences of multiple proteins, which modulates the function of monocytes and monocyte-derived pro-inflammatory macrophages and further mediates the development of several chronic diseases such as neuropsychiatric disorders and cancer[89]. Notably, the mechanisms through which hypoxia and other factors activate and regulate A3A-mediated RNA editing remain unclear.

It is well known that the polarization of proinflammatory M1 macrophages is strongly linked to microbicidal and antitumor immune responses[90,91]. Mitochondrial function, ROS, and metabolic changes could regulate macrophage polarization[92,93], and these responses are in turn regulated by complex signaling pathways, including microRNA, DNA methylation, histone modifications, and RNA editing[94]. After confirming the ability of A3A to mediate C>U RNA editing in macrophages, Shraddha[18] and her team identified an important role for A3A in transcriptomic, pro-inflammatory, and metabolic processes that drive macrophage M1 polarization. RNA-seq analysis showed that A3A upregulated the gene expression of proinflammatory cytokines, such as *TNF*, *IL-6*, and *IL-18*. Meanwhile, it also restricted glycolytic and metabolic pathways in M1 macrophages. The above findings suggest that A3A may amplify the proinflammatory responses of M1 macrophages in pathological microenvironments, but at the cost of a gene-mediated metabolic

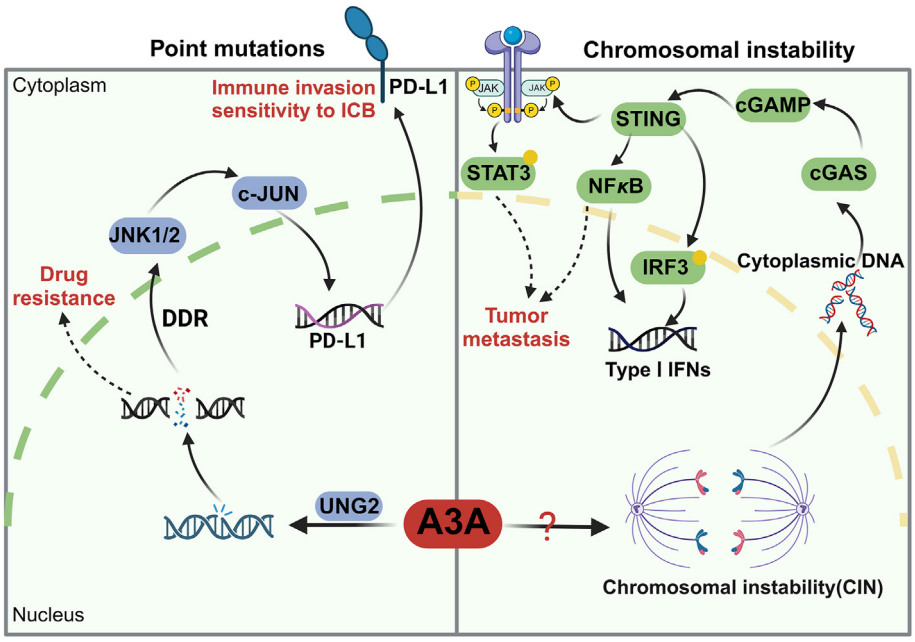


Figure 4 The function of A3A in tumor cells. A3A induces point mutations that activate the DDR-JNK/c-JUN pathway, subsequently promoting PD-L1 expression in tumor cells and increasing immune invasion sensitivity to immune checkpoint blockade. Induction of A3A in response to targeted therapies drives the evolution of drug-tolerant persistent cells. A3A also induces chromosomal instability, leading to the production of cytoplasmic DNA, which triggers the cGAS−STING pathway, promotes the expression of type I IFNs, and simultaneously activates the NF-κB and STAT3 pathways, which may drive tumor metastasis.

response, which might be mediated through its RNA editing function. These results highlight the potential for A3A to enhance macrophage-mediated host defense mechanisms by amplifying inflammation, but to be detrimental under other conditions associated with inflammatory damage (Fig. 5). Although the exact mechanism by which A3A promotes M1 polarization *via* RNA editing remains unexplored, it is speculated that such editing might influence the expression or stability of specific genes, thereby triggering a series of downstream cascading effects that promote the M1 phenotype. Whether the effect of A3A

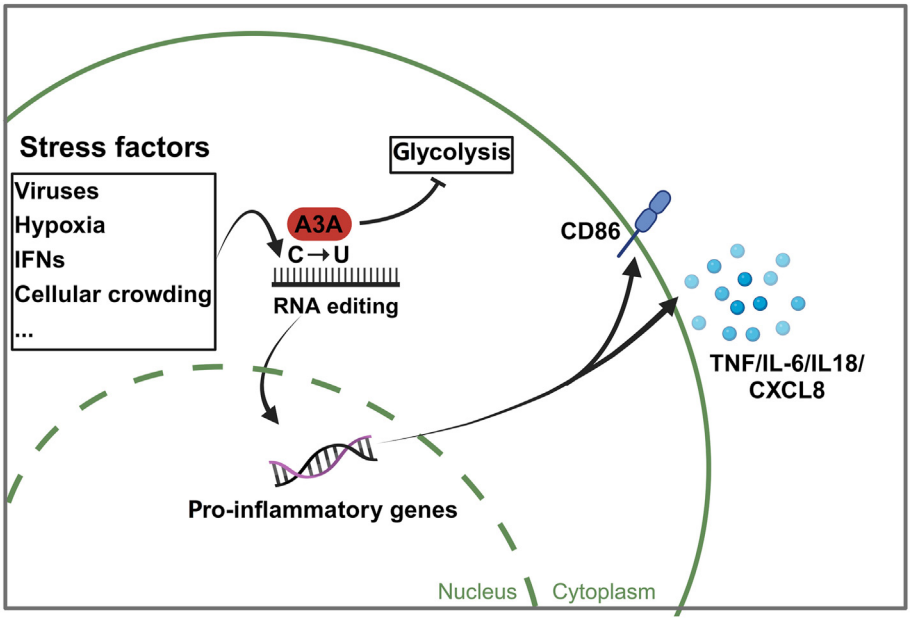


Figure 5 The role of A3A in macrophages. Certain stress factors such as virus invasion, hypoxia, IFNs, and cellular crowding in macrophages can induce A3A expression. In response, A3A inhibits glycolysis within macrophages and edits their RNA, facilitating their differentiation into a pro-inflammatory and anti-tumor phenotype.

on pro-inflammatory macrophage polarization alters their capacity to inhibit tumor growth remains an open question.

3.4. *Other mechanisms of A3A in tumorigenesis*

Multiple studies have shown that A3A can promote tumorigenesis through direct or indirect effects, suggesting that the impact of A3A on cellular physiology extends beyond its role as an editing enzyme. Niocel et al. revealed that A3A not only directly induces mutations in genomic DNA but also creates an environment conducive to tumorigenesis indirectly *via* the generation of ROS[95,96] (Fig. 3). Expression of the immune checkpoint PD-L1 has been shown to be associated with overexpression of A3 family members and A3-driven localized hypermutation[97,98]. Notably, A3A and PD-L1 are interconnected in diverse cancers, where A3A-mediated genomic mutations in conjunction with A3A-induced PD-L1 expression may contribute to tumorigenesis and an immunosuppressive tumor microenvironment (Fig. 4). Zhao et al.[17] investigated the signaling pathways of A3A in the regulation of PD-L1 and found that this regulation was dependent on the deaminase activity of A3A, independent of IFN signaling. Meanwhile, it is intricately linked to DNA replication stress and the JNK/c-JUN signaling pathway. Recently, Wörmann et al.[57] discovered that A3A promotes the progression and metastasis of pancreatic ductal adenocarcinoma in a manner independent of its deaminase activity. Instead, A3A induces chromosomal instability (CIN) rather than point mutations in the tumor cells. CIN is a distinguishing feature of human cancers characterized by erroneous chromosomal segregation, resulting in the generation of cytoplasmic dsDNA, which in turn triggers the dsDNA-sensing cGAS−STING pathway response and promotes tumor metastasis[99]. The cGAS−STING pathway initially evolved as an innate immune defense mechanism for detecting viral pathogens and exhibits acute activation during microbial infections to promote a type I IFNs response. Type I IFNs lead to enhanced MHC class I expression in tumor cells and the induction of CXCR3-binding chemokines CXCL9 and CXCL10, thus playing an important role in the generation of antitumor immunity[100]. However, the sustained and chronic activation of this pathway has been identified as a significant consequence of CIN, often involving the NF-κB pathway. Consistent with these observations, Wörmann et al. documented the production of micronuclei and cytoplasmic dsDNA in tumor cells overexpressing A3A with concomitant activation of NF-κB[57] (Fig. 4). Intriguingly, knockdown of STING effectively reversed A3A overexpression-induced tumor metastasis. These findings clearly establish that CIN production and subsequent activation of the STING pathway are critical mediators of A3A expression, thereby driving tumor metastasis.

In a pioneering study introducing the innovative CRISPRa technology, researchers employed CRISPRa to scrutinize regulators within primary human T cells upon exposure to stimulatory cytokines. Notably, several of the A3 family members, including A3A, emerged as pivotal controllers of $CD8^+$ T cells. This finding underscores the potential significance of A3A in shaping T cell function, particularly in relation to $CD8^+$ T cells[20]. Furthermore, following a comprehensive analysis of diverse databases, we uncovered a high expression of A3A in other innate immune cells, such as neutrophils and dendritic cells. Intriguingly, A3A expression was found to be highly correlated with these immune cells' penetration into specific tumor types, suggesting that A3A may exert a substantial influence on these cells, especially in anti-tumor immune responses. Our earlier research discovered a connection between lipid metabolism and A3A expression in cells[101]. Given the critical role of lipid metabolism in tumor cells, where it is harnessed to maintain rapid cell proliferation, survival, invasion, and metastasis, it is conceivable that A3A may also have an impact on tumor progression through pathways related to lipid metabolism.

4. Discussion

Extensive evidence clearly establishes that A3A, like its counterparts in the A3 family, plays a key role in antiviral defense and tumorigenesis. Firstly, A3A exerts its deaminase function against cancer-associated viruses, inducing mutations that affect the normal function of viral genes and result in the production of non-functional viral proteins. Secondly, A3A extends its deaminase effects to human genomic DNA, where recurrent low-level mutations generated by A3A can catalyze the conversion of healthy genetic sequences into oncogenes. Finally, the expression of A3A in macrophages serves to regulate their M1/M2 polarization through RNA editing. The distinctive functional polarization of tumor-associated macrophages is pivotal in determining their ability to either promote or suppress tumor growth. The different roles of A3A in different cells may be due to its intracellular localization and interactions with various effector molecules in the intracellular microenvironment (Table 2). A3A is widely distributed both in the cytoplasm and the nucleus. In the cytoplasm, A3A can limit viral replication by inducing mutations in the viral genome or by interacting with other cytoplasmic proteins to affect its functional activity and localization[31,33,63]. In the nucleus, A3A can edit genomic DNA, thereby activating DNA damage repair pathways and inducing cancer-related mutations[14,69,70,75]. There is still much research to be done to elucidate the complex roles and potential mechanisms of A3A in different microenvironments.

Given the need for rapid tumor proliferation, coupled with the chronic hypoxia and anaerobic fermentation-driven energy metabolism inherent in the tumor microenvironment, an acidic tumor environment replete with high levels of inflammatory factors may be created. Such a unique environment potentially orchestrates certain mechanisms to regulate the DNA/RNA editing process of A3A, thereby linking A3A activity to the process of tumorigenesis and progression. A3A-induced mutations in cancer cells contribute to intra-tumor heterogeneity, which is linked to T-cell exhaustion and decreased patient survival. In this case, inhibition of A3A activity can prevent tumor development, and although there is no established A3A inhibitor, we note that studies have revealed the structural basis for competitive inhibition of A3A[102-104]. These inhibitors can effectively block A3A activity in human cells and hold promise for new anti-mutagenic and anti-cancer therapies. Conversely, tumors with high genomic instability have a greater number of neoantigens, making them more responsive to immunotherapy. Additionally, leveraging the foundation of A3A-induced genetic instability in tumors may present a promising new approach to advancing tumor therapy by potentially inducing greater damage. There is a patented invention of A3A-encoded vector for the prevention and treatment of solid tumors[105], suggesting that A3A as a therapeutic target for tumors holds promise. As mentioned above, the roles of A3A in specific tumors may not be exactly the same and further studies are needed. Hence, it becomes critical to discern the dependence of A3A enzyme function on tumor or cell types. It is also imperative to recognize

Table 2 Summary of the current status of A3A functional research.

Cell type	Intracellular site of action	Mechanism	Direct result	Related pathway	Impact on tumors
Normal cell	Cytoplasm	Virus DNA/RNA editing	Viral inhibition	DDR; cGAS−STING	Tumor suppression
	Nuclear	Genome editing	Mutation accumulation	DDR	Promote tumorigenesis
Tumor cell	Nuclear	Genome editing	Genomic instability	DDR; cGAS−STING; JNK/c-JUN	Tumor suppression; Tumor metastasis; Develop drug resistance
Macrophage[a]	Cytoplasm	RNA editing	M1 polarization	Not clear	Tumor suppression

[a]A3A is specifically highly expressed in monocytes and has a unique function in such cells.

that there may be avenues that have not yet been explored. Researchers have provided preliminary descriptions of the possible roles and mechanisms of A3A in tumors through tumor database analysis, cell line construction, and other techniques. These explorations promise to offer novel directions and innovative concepts for advancing tumor research and intervention strategies.

A3A is ubiquitous in organisms and is abundantly expressed in myeloid lineage-derived cells represented by macrophages. Several mechanisms for elevated A3A expression *in vivo* have been demonstrated (Fig. 2). For instance, during viral infection, host pattern recognition receptors such as RIG-I and MDA5 are triggered, initiating a cascade of responses involving MAVS, IRF3, and STAT2, which in turn promotes A3A expression[64]. Similarly, activation of the NF-κB pathway in response to genotoxic stress leads to a transient upregulation of A3A expression[64]. However, the question arises: why does the generalized expression of A3A not affect our own cells? We hypothesize that, firstly, although A3A is expressed in both the cytoplasm and nucleus to some extent, it is primarily localized in the cytoplasm. Moreover, the overall intranuclear expression level of A3A may not reach a threshold sufficient to impact genomic DNA. Additionally, it has been reported that endogenous A3A can be chelated out of the nucleus and thus retained in the cytoplasm[106]. Given the outlined functions of A3A, it is possible that specific intracellular regulatory mechanisms exist to modulate A3A activity, thereby preventing its potentially deleterious effects on cell growth. The TRIB3 protein may prevent A3A from editing nuclear DNA by promoting the degradation of A3A and preventing A3A from being translocated to the nucleus. This action diminishes nuclear DNA editing and chromosomal DNA damage, effectively neutralizing the cytotoxic effects of A3A[106,107]. Simultaneously, research has highlighted the role that ssDNA-binding protein play in maintaining genomic stability by encasing ssDNA regions in the nucleus and preventing A3A from reaching target sequences on non-duplex DNA[108]. Moreover, there is a chaperonin-containing TCP1 in the cell that contributes to protein folding and function. It also interacts with A3A, and A3A mutation signaling is abundant in cancers with TCP1 inactivation[109]. These findings underscore the capacity of certain effector proteins to counteract A3A-induced irreversible genomic alterations. Notably, these aforementioned factors or pathways that can regulate the expression and activity of A3A, such as IFNs, the NF-κB pathway, TRIB3, etc., have been proven to be closely related to the development of cancer and are widely used in cancer therapy[100,110-112]. However, the connection between the application of these targets in cancer and the regulation of A3A expression requires more research.

A3A overexpression is present in many different types of tumors, but the causal relationship between A3A and carcinogenesis remains unclear and insufficiently substantiated. The off-target effects of A3A on the cellular genome and the RNA editing effects on intrinsic immune cells, represented by macrophages, in carcinogenesis and prognosis also need to be investigated in greater depth. Currently, the majority of research on A3A function is limited to verification in cell lines. Although cell culture is valuable, it cannot perfectly mimic the interactions and diverse physiological responses exhibited in an organism undergoing change. In order to gain a deeper understanding, it is essential to explore the phenotypic effects and mechanisms of A3A using animal models. And it is imperative to unravel the mechanisms of A3A activation, as this information enables interventions in tumorigenesis and progression by modulating the actions of A3A. In conclusion, A3A may be a potential target for tumor therapy, either by promoting the production of tumor-specific neoantigens in conjunction with immunotherapy to help immune cells kill tumors or by preventing mutations to halt the spread of cancer. The regulatory influence of A3A in immune cells, specifically macrophages, is also expected to be applied in clinical tumor therapy.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Acknowledgments

This study was supported by Shanghai Post-doctoral Excellence Program (No. 2022689, China).

Author contributions

Yuqi Yang: Writing − review & editing, Writing − original draft, Visualization, Investigation, Data curation, Conceptualization. Nan Liu: Writing − review & editing, Supervision, Investigation, Conceptualization. Likun Gong: Supervision.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, et al. The repertoire of mutational signatures in human cancer. *Nature* 2020;**578**:94−101.
2. Bergstrom EN, Luebeck J, Petljak M, Khandekar A, Barnes M, Zhang T, et al. Mapping clustered mutations in cancer reveals APOBEC3 mutagenesis of ecDNA. *Nature* 2022;**602**:510−7.
3. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SAJR, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature* 2013;**500**:415−21.
4. Chan K, Roberts SA, Klimczak LJ, Sterling JF, Saini N, Malc EP, et al. An APOBEC3A hypermutation signature is distinguishable from the signature of background mutagenesis by APOBEC3B in human cancers. *Nat Genet* 2015;**47**:1067−72.
5. Burns MB, Lackey L, Carpenter MA, Rathore A, Land AM, Leonard B, et al. APOBEC3B is an enzymatic source of mutation in breast cancer. *Nature* 2013;**494**:366−70.
6. Starrett GJ, Luengas EM, McCann JL, Ebrahimi D, Temiz NA, Love RP, et al. The DNA cytosine deaminase APOBEC3H haplotype I likely contributes to breast and lung cancer mutagenesis. *Nat Commun* 2016;**7**:12918.
7. Law EK, Levin-Klein R, Jarvis MC, Kim H, Argyris PP, Carpenter MA, et al. APOBEC3A catalyzes mutation and drives carcinogenesis *in vivo*. *J Exp Med* 2020;**217**:e20200261.
8. Cortez LM, Brown AL, Dennis MA, Collins CD, Brown AJ, Mitchell D, et al. APOBEC3A is a prominent cytidine deaminase in breast cancer. *PLoS Genet* 2019;**15**:e1008545.
9. Langenbucher A, Bowen D, Sakhtemani R, Bournique E, Wise JF, Zou L, et al. An extended APOBEC3A mutation signature in cancer. *Nat Commun* 2021;**12**:1602.
10. Ito F, Fu Y, Kao S-CA, Yang H, Chen XS. Family-wide comparative analysis of cytidine and methylcytidine deamination by eleven human APOBEC proteins. *J Mol Biol* 2017;**429**:1787−99.
11. Buisson R, Langenbucher A, Bowen D, Kwan EE, Benes CH, Zou L, et al. Passenger hotspot mutations in cancer driven by APOBEC3A and mesoscale genomic features. *Science* 2019;**364**:eaaw2872.
12. Swanton C, McGranahan N, Starrett GJ, Harris RS. APOBEC enzymes: mutagenic fuel for cancer evolution and heterogeneity. *Cancer Discov* 2015;**5**:704−12.
13. Buisson R, Lawrence MS, Benes CH, Zou L. APOBEC3A and APOBEC3B activities render cancer cells susceptible to ATR inhibition. *Cancer Res* 2017;**77**:4567−78.
14. Landry S, Narvaiza I, Linfesty DC, Weitzman MD. APOBEC3A can activate the DNA damage response and cause cell-cycle arrest. *EMBO Rep* 2011;**12**:444−50.
15. Isozaki H, Sakhtemani R, Abbasi A, Nikpour N, Stanzione M, Oh S, et al. Therapy-induced APOBEC3A drives evolution of persistent cancer cells. *Nature* 2023;**620**:393−401.
16. Li Z, He H, Ren X, Chen Y, Liu W, Pu R, et al. APOBEC3A suppresses cervical cancer *via* apoptosis. *J Cancer* 2023;**14**:3429−43.
17. Zhao K, Zhang Q, Flanagan SA, Lang X, Jiang L, Parsels LA, et al. Cytidine deaminase APOBEC3A regulates PD-L1 expression in cancer cells in a JNK/c-JUN-dependent manner. *Mol Cancer Res* 2021;**19**:1571−82.
18. Alqassim EY, Sharma S, Khan ANMNH, Emmons TR, Cortes Gomez E, Alahmari A, et al. RNA editing enzyme APOBEC3A promotes pro-inflammatory M1 macrophage polarization. *Commun Biol* 2021;**4**:102.
19. Sharma S, Patnaik SK, Thomas Taggart R, Kannisto ED, Enriquez SM, Gollnick P, et al. APOBEC3A cytidine deaminase induces RNA editing in monocytes and macrophages. *Nat Commun* 2015;**6**:6881.
20. Schmidt R, Steinhart Z, Layeghi M, Freimer JW, Bueno R, Nguyen VQ, et al. CRISPR activation and interference screens decode stimulation responses in primary human T cells. *Science* 2022;**375**:eabj4008.
21. Jarmuz A, Chester A, Bayliss J, Gisbourne J, Dunham I, Scott J, et al. An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. *Genomics* 2002;**79**:285−96.
22. Navarro F, Bollman B, Chen H, König R, Yu Q, Chiles K, et al. Complementary function of the two catalytic domains of APOBEC3G. *Virology* 2005;**333**:374−86.
23. Wedekind JE, Gillilan R, Janda A, Krucinska J, Salter JD, Bennett RP, et al. Nanostructures of APOBEC3G support a hierarchical assembly model of high molecular mass ribonucleoprotein particles from dimeric subunits. *J Biol Chem* 2006;**281**:38122−6.
24. LaRue RS, Jónsson SR, Silverstein KA, Lajoie M, Bertrand D, El-Mabrouk N, et al. The artiodactyl APOBEC3 innate immune repertoire shows evidence for a multi-functional domain organization that existed in the ancestor of placental mammals. *BMC Mol Biol* 2008;**9**:104.
25. Zhang H, Yang B, Pomerantz RJ, Zhang C, Arunachalam SC, Gao L. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* 2003;**424**:94−8.
26. Zhai C, Ma L, Zhang Z, Ding J, Wang J, Zhang Y, et al. Identification and characterization of loop7 motif and its role in regulating biological function of human APOBEC3G through molecular modeling and biological assay. *Acta Pharm Sin B* 2017;**7**:571−82.
27. Bogerd HP, Wiegand HL, Hulme AE, Garcia-Perez JL, O'Shea KS, Moran JV, et al. Cellular inhibitors of long interspersed element 1 and Alu retrotransposition. *Proc Natl Acad Sci USA* 2006;**103**:8780−5.
28. Chen H, Lilley CE, Yu Q, Lee DV, Chou J, Narvaiza I, et al. APOBEC3A is a potent inhibitor of adeno-associated virus and retrotransposons. *Curr Biol* 2006;**16**:480−5.
29. Kinomoto M, Kanno T, Shimura M, Ishizaka Y, Kojima A, Kurata T, et al. All APOBEC3 family proteins differentially inhibit LINE-1 retrotransposition. *Nucleic Acids Res* 2007;**35**:2955−64.
30. Narvaiza I, Linfesty DC, Greener BN, Hakata Y, Pintel DJ, Logue E, et al. Deaminase-independent inhibition of parvoviruses by the APOBEC3A cytidine deaminase. *PLoS Pathog* 2009;**5**:e1000439.
31. Goila-Gaur R, Khan MA, Miyagi E, Kao S, Strebel K. Targeting APOBEC3A to the viral nucleoprotein complex confers antiviral activity. *Retrovirology* 2007;**4**:61.
32. Koning FA, Goujon C, Bauby H, Malim MH. Target cell-mediated editing of HIV-1 cDNA by APOBEC3 proteins in human macrophages. *J Virol* 2011;**85**:13448−52.
33. Berger G, Durand S, Fargier G, Nguyen X-N, Cordeil S, Bouaziz S, et al. APOBEC3A is a specific inhibitor of the early phases of HIV-1 infection in myeloid cells. *PLoS Pathog* 2011;**7**:e1002221.
34. Bishop KN, Holmes RK, Malim MH. Antiviral potency of APOBEC proteins does not correlate with cytidine deamination. *J Virol* 2006;**80**:8450−8.
35. Aguiar RS, N L, Tanuri A, Peterlin BM. Vpr.A3A chimera inhibits HIV replication. *J Biol Chem* 2008;**283**:2518−25.
36. Bulliard Y, Narvaiza I, Bertero A, Peddi S, Rohrig UF, Ortiz M, et al. Structure−function analyses point to a polynucleotide-accommodating groove essential for APOBEC3A restriction activities. *J Virol* 2011;**85**:1765−76.
37. Stenglein MD, Burns MB, Li M, Lengyel J, Harris RS. APOBEC3 proteins mediate the clearance of foreign DNA from human cells. *Nat Struct Mol Biol* 2010;**17**:222−9.
38. Vartanian JP, Guétard D, Henry M, Wain-Hobson S. Evidence for editing of human papillomavirus DNA by APOBEC3 in benign and precancerous lesions. *Science* 2008;**320**:230−3.
39. Pham P, Landolph A, Mendez C, Li N, Goodman MF. A biochemical analysis linking APOBEC3A to disparate HIV-1 restriction and skin cancer. *J Biol Chem* 2013;**288**:29294−304.
40. Doehle BP, Schäfer A, Wiegand HL, Bogerd HP, Cullen BR. Differential sensitivity of murine leukemia virus to APOBEC3-mediated inhibition is governed by virion exclusion. *J Virol* 2005;**79**:8201−7.
41. Bishop KN, Holmes RK, Sheehy AM, Davidson NO, Cho SJ, Malim MH. Cytidine deamination of retroviral DNA by diverse APOBEC proteins. *Curr Biol* 2004;**14**:1392−6.

42. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, et al. Mutational processes molding the genomes of 21 breast cancers. *Cell* 2012;**149**:979−93.
43. Smith NJ, Fenton TR. The APOBEC3 genes and their role in cancer: insights from human papillomavirus. *J Mol Endocrinol* 2019;**62**: R269−87.
44. MacMillan AL, Kohli RM, Ross SR. APOBEC3 inhibition of mouse mammary tumor virus infection: the role of cytidine deamination *versus* inhibition of reverse transcription. *J Virol* 2013;**87**:4808−17.
45. Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, et al. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. *Nat Genet* 2013;**45**:970−6.
46. Mas-Ponte D, Supek F. DNA mismatch repair promotes APOBEC3-mediated diffuse hypermutation in human cancers. *Nat Genet* 2020; **52**:958−68.
47. DeWeerd RA, Németh E, Póti Á, Petryk N, Chen C-L, Hyrien O, et al. Prospectively defined patterns of APOBEC3A mutagenesis are prevalent in human cancers. *Cell Rep* 2022;**38**:110555.
48. Pyeon D, Pearce SM, Lank SM, Ahlquist P, Lambert PF, Münger K. Establishment of human papillomavirus infection requires cell cycle progression. *PLoS Pathog* 2009;**5**:e1000318.
49. Naumann JA, Argyris PP, Carpenter MA, Gupta HB, Chen Y, Temiz NA, et al. DNA deamination is required for human APOBEC3A-driven hepatocellular carcinoma *in vivo*. *Int J Mol Sci* 2023; **24**:9305.
50. Petljak M, Dananberg A, Chu K, Bergstrom EN, Striepen J, von Morgen P, et al. Mechanisms of APOBEC3 mutagenesis in human cancer cells. *Nature* 2022;**607**:799−807.
51. Kim YS, Sun DS, Yoon JS, Ko YH, Won HS, Kim JS. Clinical implications of APOBEC3A and 3B expression in patients with breast cancer. *PLoS One* 2020;**15**:e0230261.
52. Zhang M, Wei Z, Zhao H, Zhang S, Wu J, Zhou J, et al. The role of APOBEC3A in cervical cancer development and progression: a retrospective study. *Drug Discov Ther* 2023;**17**:191−200.
53. Kostrzak A, Caval V, Escande M, Pliquet E, Thalmensi J, Bestetti T, et al. APOBEC3A intratumoral DNA electroporation in mice. *Gene Ther* 2017;**24**:74−83.
54. Kondo S, Wakae K, Wakisaka N, Nakanishi Y, Ishikawa K, Komori T, et al. APOBEC3A associates with human papillomavirus genome integration in oropharyngeal cancers. *Oncogene* 2017;**36**: 1687−97.
55. Xu F, Liu T, Zhou Z, Zou C, Xu S. Comprehensive analyses identify APOBEC3A as a genomic instability-associated immune prognostic biomarker in ovarian cancer. *Front Immunol* 2021;**12**:749369.
56. Liang JW, Shi ZZ, Shen TY, Che X, Wang Z, Shi SS, et al. Identification of genomic alterations in pancreatic cancer using array-based comparative genomic hybridization. *PLoS One* 2014;**9**:e114616.
57. Wörmann SM, Zhang A, Thege FI, Cowan RW, Rupani DN, Wang R, et al. APOBEC3A drives deaminase domain-independent chromosomal instability to promote pancreatic cancer metastasis. *Nat Cancer* 2021;**2**:1338−56.
58. Heller M, Prigge ES, Kaczorowski A, von Knebel Doeberitz M, Hohenfellner M, Duensing S. APOBEC3A expression in penile squamous cell carcinoma. *Pathobiology* 2017;**85**:169−78.
59. Warren CJ, Santiago ML, Pyeon D. APOBEC3: friend or foe in human papillomavirus infection and oncogenesis?. *Annu Rev Virol* 2022;**9**:375−95.
60. Warren CJ, Xu T, Guo K, Griffin LM, Westrich JA, Lee D, et al. APOBEC3A functions as a restriction factor of human papillomavirus. *J Virol* 2014;**89**:688−702.
61. Warren CJ, Van Doorslaer K, Pandey A, Espinosa JM, Pyeon D. Role of the host restriction factor APOBEC3 on papillomavirus evolution. *Virus Evol* 2015;**1**:vev015.
62. Warren CJ, Pyeon D. APOBEC3 in papillomavirus restriction, evolution and cancer progression. *Oncotarget* 2015;**6**:39385−6.
63. Chen S, Li X, Qin J, Chen Y, Zhang D. APOBEC3A possesses anticancer and antiviral effects by differential inhibition of HPV E6 and E7 expression on cervical cancer. *Int J Clin Exp Med* 2015;**8**: 10548−57.
64. Oh S, Bournique E, Bowen D, Jalili P, Sanchez A, Ward I, et al. Genotoxic stress and viral infection induce transient expression of APOBEC3A and pro-inflammatory genes through two distinct pathways. *Nat Commun* 2021;**12**:4917.
65. Bonvin M, Achermann F, Greeve I, Stroka D, Keogh A, Inderbitzin D, et al. Interferon-inducible expression of APOBEC3 editing enzymes in human hepatocytes and inhibition of hepatitis B virus replication. *Hepatology* 2006;**43**:1364−74.
66. Chang J, Guo F, Zhao X, Guo JT. Therapeutic strategies for a functional cure of chronic hepatitis B virus infection. *Acta Pharm Sin B* 2014;**4**:248−57.
67. Scholtés GK, Sawyer AM, Vaca CC, Clerc I, Roh M, Song C, et al. The von Hippel−Lindau Cullin-RING E3 ubiquitin ligase regulates APOBEC3 cytidine deaminases. *Transl Res* 2021;**237**:1−15.
68. Zhou L, Ren JH, Cheng ST, Xu HM, Chen WX, Chen DP, et al. A functional variant in ubiquitin conjugating enzyme E2 L3 contributes to hepatitis B virus infection and maintains covalently closed circular DNA stability by inducing degradation of apolipoprotein B mRNA editing enzyme catalytic subunit 3A. *Hepatology* 2019;**69**: 1885−902.
69. Suspene R, Aynaud MM, Guetard D, Henry M, Eckhoff G, Marchio A, et al. Somatic hypermutation of human mitochondrial and nuclear DNA by APOBEC3 cytidine deaminases, a pathway for DNA catabolism. *Proc Natl Acad Sci USA* 2011;**108**:4858−63.
70. Green AM, Landry S, Budagyan K, Avgousti DC, Shalhout S, Bhagwat AS, et al. APOBEC3A damages the cellular genome during DNA replication. *Cell Cycle* 2016;**15**:998−1008.
71. Taylor BJ, Nik-Zainal S, Wu YL, Stebbings LA, Raine K, Campbell PJ, et al. DNA deaminases induce break-associated mutation showers with implication of APOBEC3B and 3A in breast cancer kataegis. *Elife* 2013;**2**:e00534.
72. Henderson S, Fenton T. APOBEC3 genes: retroviral restriction factors to cancer drivers. *Trends Mol Med* 2015;**21**:274−84.
73. Suspène R, Mussil B, Laude H, Caval V, Berry N, Bouzidi MS, et al. Self-cytoplasmic DNA upregulates the mutator enzyme APOBEC3A leading to chromosomal DNA damage. *Nucleic Acids Res* 2017;**45**: 3231−41.
74. Hoopes James I, Cortez Luis M, Mertz Tony M, Malc Ewa P, Mieczkowski Piotr A, Roberts Steven A. APOBEC3A and APOBEC3B preferentially deaminate the lagging strand template during DNA replication. *Cel Rep* 2016;**14**:1273−82.
75. Mussil B, Suspène R, Aynaud MM, Gauvrit A, Vartanian JP, Wain-Hobson S. Human APOBEC3A isoforms translocate to the nucleus and induce DNA double strand breaks leading to cell stress and death. *PLoS One* 2013;**8**:e73641.
76. Petljak M, Chu K, Dananberg A, Bergstrom EN, Morgen Pv, Alexandrov LB, et al. The APOBEC3A deaminase drives episodic mutagenesis in cancer cells. *bioRxiv* 2021. Available from: https://doi.org/10.1101/2021.02.14.431145.
77. Jalili P, Bowen D, Langenbucher A, Park S, Aguirre K, Corcoran RB, et al. Quantification of ongoing APOBEC3A activity in tumor cells by monitoring RNA editing at hotspots. *Nat Commun* 2020;**11**:2971.
78. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* 2019;**51**: 202−6.
79. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol* 2019;**30**:44−56.
80. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med* 2014;**373**:1984.
81. Nik-Zainal S, Wedge DC, Alexandrov LB, Petljak M, Butler AP, Bolli N, et al. Association of a germline copy number polymorphism

of APOBEC3A and APOBEC3B with burden of putative APOBEC-dependent mutations in breast cancer. *Nat Genet* 2014;**46**:487−91.
82. Caval V, Suspène R, Shapira M, Vartanian JP, Wain-Hobson S. A prevalent cancer susceptibility APOBEC3A hybrid allele bearing APOBEC3B 3'UTR enhances chromosomal DNA damage. *Nat Commun* 2014;**5**:5129.
83. Laskin DL. Macrophages and inflammatory mediators in chemical toxicity: a battle of forces. *Chem Res Toxicol* 2009;**22**:1376−85.
84. Zhang X, Mosser DM. Macrophage activation by endogenous danger signals. *J Pathol* 2007;**214**:161−78.
85. Orenstein JM, Meltzer MS, Phipps T, Gendelman HE. Cytoplasmic assembly and accumulation of human immunodeficiency virus types 1 and 2 in recombinant human colony-stimulating factor-1-treated human monocytes: an ultrastructural study. *J Virol* 1988;**62**:2578−86.
86. Orenstein JM, Fox C, Wahl SM. Macrophages as a source of HIV during opportunistic infections. *Science* 1997;**276**:1857−61.
87. Peng G, Greenwell-Wild T, Nares S, Jin W, Lei KJ, Rangel ZG, et al. Myeloid differentiation and susceptibility to HIV-1 are linked to APOBEC3 expression. *Blood* 2007;**110**:393−400.
88. Bogerd HP, Wiegand HL, Doehle BP, Lueders KK, Cullen BR. APOBEC3A and APOBEC3B are potent inhibitors of LTR-retrotransposon function in human cells. *Nucleic Acids Res* 2006;**34**:89−95.
89. Tang W, Fei Y, Page M. Biological significance of RNA editing in cells. *Mol Biotechnol* 2012;**52**:91−100.
90. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. *J Leukoc Biol* 2006;**80**:1298−307.
91. Van Ginderachter JA, Movahedi K, Hassanzadeh Ghassabeh G, Meerschaut S, Beschin A, Raes G, et al. Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion. *Immunobiology* 2006;**211**:487−501.
92. O'Neill LAJ, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med* 2016;**213**:15−23.
93. Mills EL, Kelly B, Logan A, Costa ASH, Varma M, Bryant CE, et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell* 2016;**167**:457−70.
94. Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. *Nat Rev Immunol* 2011;**11**:750−61.
95. Niocel M, Appourchaux R, Nguyen X-N, Delpeuch M, Cimarelli A. The DNA damage induced by the cytosine deaminase APOBEC3A leads to the production of ROS. *Sci Rep* 2019;**9**:4714.
96. Idelchik MDPS, Begley U, Begley TJ, Melendez JA. Mitochondrial ROS control of cancer. *Semin Cancer Biol* 2017;**47**:57−66.
97. Mullane SA, Werner L, Rosenberg J, Signoretti S, Callea M, Choueiri TK, et al. Correlation of Apobec mrna expression with overall survival and pd-l1 expression in urothelial carcinoma. *Sci Rep* 2016;**6**:27702.
98. Boichard A, Tsigelny IF, Kurzrock R. High expression of PD-1 ligands is associated with kataegis mutational signature and APOBEC3 alterations. *OncoImmunology* 2017;**6**:e1284719.
99. Bakhoum SF, Ngo B, Laughney AM, Cavallo JA, Murphy CJ, Ly P, et al. Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* 2018;**553**:467−72.
100. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. *Nat Rev Immunol* 2015;**15**:405−14.
101. Liu N, Guo YN, Wang XJ, Ma J, He YT, Zhang F, et al. Copy number analyses identified a novel gene: APOBEC3A related to lipid metabolism in the pathogenesis of preeclampsia. *Front Cardiovasc Med* 2022;**9**:841249.
102. Serrano JC, von Trentini D, Berríos KN, Barka A, Dmochowski IJ, Kohli RM. Structure-guided design of a potent and specific inhibitor against the genomic mutator APOBEC3A. *ACS Chem Biol* 2022;**17**:3379−88.
103. Harjes S, Kurup HM, Rieffer AE, Bayarjargal M, Filitcheva J, Su Y, et al. Structure-guided inhibition of the cancer DNA-mutating enzyme APOBEC3A. *Nat Commun* 2023;**14**:6382.
104. King JJ, Borzooee F, Im J, Asgharpour M, Ghorbani A, Diamond CP, et al. Structure-based design of first-generation small molecule inhibitors targeting the catalytic pockets of AID, APOBEC3A, and APOBEC3B. *ACS Pharmacol Transl Sci* 2021;**4**:1390−407.
105. Pierre LD, Anna K, Simon WH. inventors; Invectys, assignee. APOBEC3A as an anti-tumor agent. US patent EP3003359. 2018-11-07.
106. Land AM, Law EK, Carpenter MA, Lackey L, Brown WL, Harris RS. Endogenous APOBEC3A DNA cytosine deaminase is cytoplasmic and nongenotoxic. *J Biol Chem* 2013;**288**:17253−60.
107. Aynaud MM, Suspène R, Vidalain PO, Mussil B, Guétard D, Tangy F, et al. Human Tribbles 3 protects nuclear DNA from cytidine deamination by APOBEC3A. *J Biol Chem* 2012;**287**:39182−92.
108. Mitra M, Hercík K, Byeon IJ, Ahn J, Hill S, Hinchee-Rodriguez K, et al. Structural determinants of human APOBEC3A enzymatic and nucleic acid binding properties. *Nucleic Acids Res* 2013;**42**:1095−110.
109. Green AM, DeWeerd RA, O'Leary DR, Hansen AR, Hayer KE, Kulej K, et al. Interaction with the CCT chaperonin complex limits APOBEC3A cytidine deaminase cytotoxicity. *EMBO Rep* 2021;**22**:e52145.
110. Platanias LC. Interferons and their antitumor properties. *J Interferon Cytokine Res* 2013;**33**:143−4.
111. Lalle G, Twardowski J, Grinberg-Bleyer Y. NF-κB in cancer immunity: friend or foe?. *Cells* 2021;**10**:355.
112. Yu JM, Sun W, Wang ZH, Liang X, Hua F, Li K, et al. TRIB3 supports breast cancer stemness by suppressing FOXO1 degradation and enhancing SOX2 transcription. *Nat Commun* 2019;**10**:5720.