SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 12: 5344-5348, 2016

APOBEC3B expression in human leptomeninges and meningiomas

MAHLON D. JOHNSON[1], JAY E. REEDER[2] and MARY O'CONNELL[1]

[1]Department of Pathology, Division of Neuropathology, and [2]Department of Urology, University of Rochester Medical Center, University of Rochester School of Medicine and Dentistry, Rochester, NY 14623, USA

Received January 11, 2016; Accepted September 15, 2016

DOI: 10.3892/ol.2016.5377

Abstract. Nucleic acid-editing enzymes of the apolipoprotein B mRNA-editing enzyme (APOBEC) family have been associated with somatic mutation in cancer. However, the role of APOBEC catalytic subunit 3B (APOBEC3B) editing in the pathogenesis of base substitutions in meningiomas is unknown. In the present study, the expression of APOBEC3B was examined by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analyses in five fetal and one adult human leptomeninges and 38 meningiomas. Genomic DNA was sequenced using the Illumina Tru-Seq Cancer Panel. Three meningioma primary cultures were also established and treated with cerebrospinal fluid form patients without neurological disease or platelet-derived growth factor-BB (PDGF-BB), prior to evaluation of APOBEC3B expression. By western blotting, APOBEC3B was revealed to be present in 100% of the fetal leptomeninges, and in 88% of World Health Organization grade I, 100% of grade II and 83% of grade III meningiomas tested, but was not different between grades. RT-qPCR revealed no difference in the mRNA expression of APOBEC3B between grades. Sequencing revealed no elevated levels of the C>T mutations that are characteristic of APOBEC3B editing of genomic DNA. Treatment with cerebrospinal fluid and PDGF-BB had no effect on APOBEC3B protein expression in the leptomeningeal or meningioma cells. These findings suggest that the mutations associated with increased APOBEC3B expression may not be central to the pathogenesis of meningiomas.

Correspondence to: Dr Mahlon D. Johnson, Department of Pathology, Division of Neuropathology, University of Rochester Medical Center, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Box 626, Rochester, NY 14623, USA
E-mail: mahlon_johnson@urmc.rochester.edu

Key words: APOBEC, arachnoid granulations, leptomeninges, meningioma

Introduction

Recent studies have indicated that normal cellular enzymatic activity may contribute to the genomic changes associated with various neoplasias (1-3). Apolipoprotein B mRNA-editing enzymes (APOBEC) comprise a family of enzymes that protect immune function and are involved in mRNA editing; their cytosine deaminase activity may also induce base substitutions in the genomes of malignancies (1,2).

APOBEC3B is a cytosine deaminase that is responsible for the deamination of cytosines in the genome of host cells, producing cytosine to thymidine mutations (2). Recently studies have found that APOBEC3B is overexpressed in several types of malignancy, resulting in clusters of C>T mutations that are considered to be the signature of APOBEC mutation in tumors. Analysis of whole-genome and whole-exome sequencing data from various types of malignancy have implicated APOBEC members in mRNA editing and the production of cytosine mutation clusters in the development or progression of numerous neoplastic processes, encompassing lung, breast, hepatic and hematopoietic malignancies (4-9). APOBEC3B overexpression has been identified in breast, head and neck cancers (7-10). Although APOBEC3B expression has not been studied in meningiomas, the presence of C>T point mutations in some meningioma cases raises the possibility that APOBEC3B may participate in the pathogenesis of at least a subset of meningiomas (11).

The present study evaluated APOBEC3B expression in addition to mutations in a series of normal leptomeninges, and World Health Organization (WHO) grade I, II and III meningiomas.

Materials and methods

Human leptomeningeal and meningioma tissue. Frozen tissues from 5 fetal and 1 adult leptomeninges and 38 meningiomas, including 17 WHO grade I, 17 grade II and 6 grade III meningiomas (12), were collected at the University of Rochester Medical Center (Rochester, NY, USA), or obtained primarily from the Cooperative Human Tissue Network (Philadelphia, PA, USA), between January 2007 and December 2014, without particular selection criteria (Table I).

Human leptomeningeal and meningioma cell cultures. Primary leptomeningeal cultures were established from two 20-week and one 22-week de-identified human fetuses ~5 h after mortality from non-neurological disease. These were minced and grown in petri dishes. Meningioma cell cultures were established from fragments from the center of two WHO grade I and one grade II meningiomas. Tissues were minced prior to plating in flasks. These tissues along with the cerebrospinal fluid were collected with the approval of the Institutional Review Board of the University of Rochester. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc.), which is insufficient for the survival and proliferation of normal glial and endothelial cells. The cells used were screened according to procedures described previously, including the detection of a leptomeningeal marker, epithelial membrane antigen (EMA) (13). For experiments, only early passages (passages 2 to 5) were used of the meningioma cell cultures that exhibited EMA immunoreactivity by immunocytochemistry (11). For immunocytochemical characterization, meningioma cell cultures were plated onto 2-well microscope slides (Nalgene NUNC International, Rochester, NY, USA) for 1 day in DMEM with 10% FBS, prior to fixation in formalin. Immunocytochemistry was performed using a mouse monoclonal antibody against EMA (prediluted; Clone 29; #N1504; Dako, Carpenteria, CA, USA) and visualized using the streptavidin-biotin-horseradish peroxidase method (Dako). Extensive cytoplasmic immunoreactivity was considered positive.

Human cerebrospinal fluid from patients without neurological disease. As meningiomas are bathed in cerebrospinal fluid, which has been demonstrated to affect meningioma cell proliferation (13), its effects on APOBEC3B were evaluated in the current study. Remnant, discarded lumbar cerebrospinal fluid was collected at the University of Rochester Medical Center and sent to the Department of Pathology between March 2009 and September 2012. Samples from patients who were found to be free of neurological disease, and who had a cytopathological analysis showing no erythrocytes or increased lymphocytes were classified as 'normal'. In patients who had cerebrospinal fluid collected as part of a staging protocol for a peripheral lymphoma, only cerebrospinal fluid from those with no lymphoma cells or abnormal numbers of lymphocytes by cytology and no neurological disease were used. In some cases, due to limited volumes, multiple samples were combined to achieve quantities sufficient for the experimental design. Cerebrospinal fluid samples were initially frozen at -17˚C prior to storage at -80˚C.

Western blot analysis of APOBEC3B protein expression in human leptomeningeal and meningioma tissues. For western blot analysis, meningioma lysates were obtained by mechanical homogenization in Upstate RIPA Lysis Buffer (EMD Millipore, Billerica, MA, USA) with 1:100 Protease Inhibitor Cocktail (Sigma-Aldrich; EMD Millipore) then frozen at -85˚C. The tissue slurries were thawed on ice and vortexed vigorously, prior to the removal of solids by centrifugation at 4˚C. Protein concentrations were quantified using a Bradford assay (Bio-Rad Protein Assay; Bio-Rad Laboratories, Inc., Hercules, CA, USA), and 30-50 μg of protein from each sample was loaded and run on a 7.5% acrylamide gel, before transfer to 0.45-μm nitrocellulose membrane. The membrane was blocked for 1 h in 5% milk in Tris-Cl buffer with Tween 20, then reacted with a polyclonal affinity-purified primary antibody against APOBEC3B (#sc-130955; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4˚C. This was followed by horseradish peroxidase-conjugated secondary antibody treatment (#170-6515; Bio-Rad Laboratories, Inc.). Detection was achieved using Clarity Western ECL Substrate (Bio-Rad Laboratories, Inc.) and imaging was performed with Bio-Rad ChemiDoc instrumentation and Image Lab software (Bio-Rad Laboratories, Inc.). Loading was assessed using a polyclonal rabbit antibody against actin (dilution, 1:3000; #4967; Cell Signaling Technology, Inc., Danvers, MA, USA); to evaluate the results, the APOBEC3B band intensity was normalized relative to that of actin.

Analysis of APOBEC3B mRNA in leptomeningeal tissue and meningioma tumors. RNA was isolated from 2 fetal and 1 adult leptomeninges, 9 WHO grade I, 10 WHO grade II and 4 WHO grade III meningiomas using an RNeasy Plus universal (#7304; Qiagen, Inc., Valencia, CA, USA) according to manufacturers specifications. Reverse transcription (RT) was performed in a 20-μl reaction mixture containing of 1 μg of RNA, using an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc.); the RT conditions were 5 min at 25˚C, 30 min at 42˚C, 5 min at 85˚C, and an indefinite hold at 4˚C. The resulting cDNA was diluted to 200 μl with water.

Quantitative polymerase chain reaction (qPCR) for APOBEC3B was performed in triplicate using primers for APOBEC3B (forward, 5'-GACCCTTTGGTCCTTCGAC-3'; reverse, 5'-GCACAGCCCCAGGAGAAG-3') and the RPL13A control gene (forward 5'-AGATGGCGGAGGTGCAG-3' and reverse 5'-GCCAGAAATGTTGATGCCTT-3'). The qPCR reaction mixture was composed of 1X iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc.), 1 μM primer mix, and 4.9 μl of diluted cDNA in a total volume of 10 μl. Reactions were performed on the Bio-Rad CFX Connect Real-Time System using the default protocol CFX_2StepAmp+Melt, as follows: 95˚C for 3 min, followed by 40 cycles of 95˚C for 10 sec and 55˚C for 30 sec, and 95˚C for 10 sec. Data was collected using Bio-Rad CFX Manager 3.0 software (Bio-Rad Laboratories, Inc.). Relative quantification was performed using the $2^{-\Delta\Delta Cq}$ calculation method (14).

Sequencing of APOBEC3B DNA in leptomeningeal tissue and meningioma tumors. DNA was isolated from frozen meningioma tissue using the Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's guidelines, and stored at -80˚C. Sequencing was performed using a TruSeq Amplicon-Cancer Panel (Illumina, Inc., San Diego, CA, USA). Sequences and controls were compared against the known sequence.

Effects of platelet-derived growth factor-BB (PDGF-BB) and cerebrospinal fluid on APOBEC3B protein expression. PDGF-BB and cerebrospinal fluid were previously demonstrated to influence leptomeningeal and meningioma cell proliferation (13). Consequently, confluent cells from

Table I. Summary of meningioma and leptomeningeal tissues used in the present study.

A, Tissues used in western blot analysis

Tissue subtype	n (%)	Mean patient age	Patient genders
Leptomeninges	6		
Fetal	5	-	U
Adult	1	40 years	M
WHO grade I meningioma	17	57 years	14 F, 3 M
Meningothelial	7 (41)		
Transitional	7 (41)		
Fibrous	3 (18)		
WHO grade II meningioma	15	53 years	12 F, 3 M
Meningothelial	6 (40)		
Transitional	3 (20)		
Fibrous	5 (33)		
Microcystic	1 (7)		
WHO grade III meningioma	6	61 years	4 F, 2 M
Anaplastic	3 (50)		
Transitional	1 (17)		
Fibrous	1 (17)		
Papillary	1 (17)		

B, Tissues used in polymerase chain reaction analysis

Tissue subtype	n (%)	Mean patient age	Patient genders
Leptomeninges	3		
Fetal	2	18 weeks	U
Adult	1	67 years	1 F
WHO grade I meningioma	9	61 years	7 F, 2 M
Meningothelial	6 (67)		
Transitional	3 (33)		
WHO grade II meningioma	10	60 years	6 F, 4 M
Meningothelial	3 (30)		
Transitional	1 (10)		
Fibrous	4 (40)		
Secretory	1 (10)		
Microcystic	1 (10)		
WHO grade III meningioma	4	64 years	3 F, 1 M
Anaplastic	3 (75)		
Papillary	1 (25)		

C, Sources of leptomeningeal and meningioma cells used for primary cultures

Type	Patient age/gender	Location	Classification/grade
LC1	20 weeks/U	Convexity	Leptomeninges
LC2	20 weeks/U	Convexity	Leptomeninges
LC3	22 weeks/U	Convexity	Leptomeninges
MC1	84 years/M	Right frontal	Meningothelial/I
MC2	65 years/F	Clivus	Meningothelial/I
MC3	80 years/M	Left frontal	Transitional/II

WHO, World Health Organization; U, unknown/unavailable; F, female; M, male; LC, leptomeningeal cells; MC, meningioma cells.

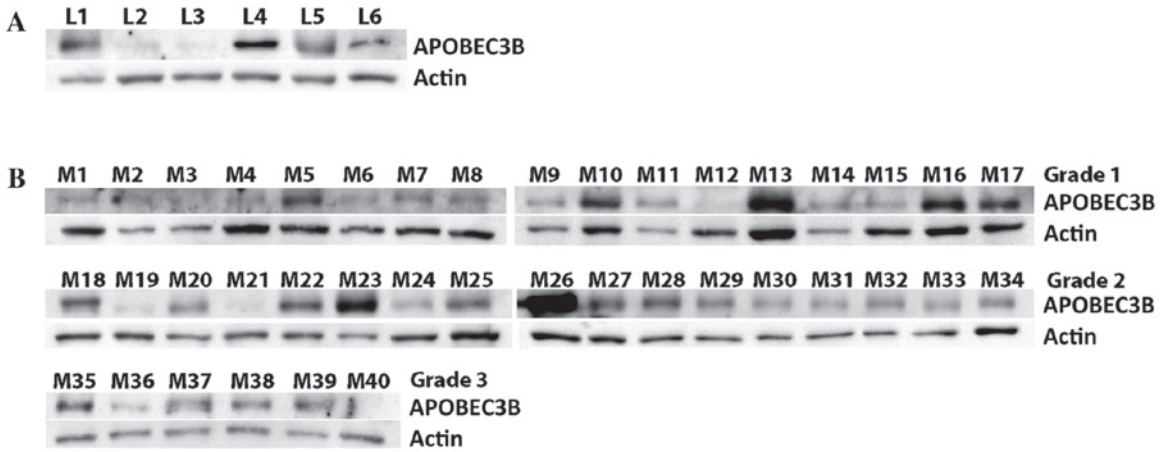

Figure 1. APOBEC3B protein expression in fetal and adult leptomeninges, and in WHO grade I, II and III meningiomas. (A) Western blot analysis of APOBEC3B adult (L1) and fetal (L2-6) leptomeningeal tissues. (B) 15 of 17 WHO grade I (M1-M17), 17 of 17 grade II (M18-34), 6 grade III (M35-M40) meningiomas showing APOBEC3B and actin bands. APOBEC3B was detected in meningiomas regardless of subtype, but without definite differences.

3 primary fetal leptomeningeal cell cultures (gestational ages 20, 20 and 22 weeks), 2 WHO grade I and 2 WHO grade II primary meningioma cultures were serum-deprived overnight prior to treatment with serum-free DMEM, DMEM containing 10 ng/ml PDGF-BB, or pooled cerebrospinal fluid from patients, for 72 h. Lysates of the cells were then analyzed by western blotting, as follows. Meningioma lysates were homogenized in RIPA Lysis Buffer as described above. Following quantification of protein concentrations, 10 μg protein from each sample was loaded and run on a 7.5% acrylamide gel, then transferred to a 0.45-μm nitrocellulose membrane. The membrane was blocked for 1 h in 5% milk in Tris-Cl buffer with Tween 20, then reacted with the affinity-purified primary antibody overnight at 4°C. This was followed by horseradish peroxidase-conjugated secondary antibody treatment. Detection was achieved with Western Lightning reagent (PerkinElmer, Inc., Waltham, MA, USA) on Kodak Xomat film. All western blots were subsequently repeated and quantified with Clarity Western ECL substrate (Bio-Rad Laboratories, Inc.) and Chemidoc software.

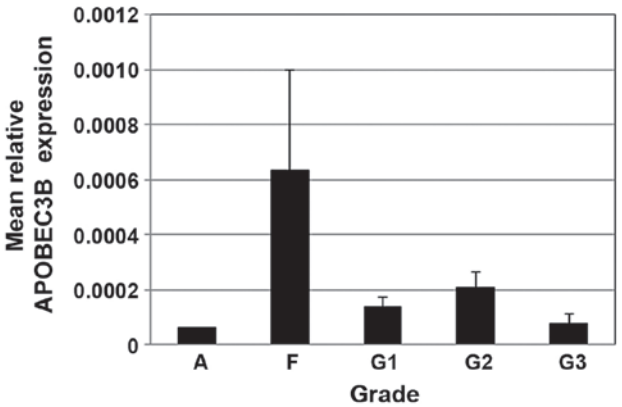

Figure 2. Relative APOBEC3B mRNA expression in fetal and adult leptomeninges and meningiomas. Reverse transcription-quantitative polymerase chain reaction analyses revealed APOBEC3B mRNA levels in fetal leptomeninges, adult leptomeninges, and in WHO grade I, II and III meningiomas; no significant differences were observed between the groups. A, adult leptomeninges; F, fetal leptomeninges; G1, WHO grade I; G2, WHO grade II; G3, WHO grade III.

Results

APOBEC3B protein expression in leptomeninges and meningioma. As shown in Fig. 1A, APOBEC3B was detected in the 16-, 17-, 20-, 22- and 23-week fetal and the adult leptomeningeal tissues. In the meningiomas, APOBEC3B was detected in 15 of 17 WHO grade I meningiomas (Fig 1B). APOBEC3B was also detected in all 17 WHO grade II and 5 of 6 grade III tumors (Fig. 1B).

Analysis of APOBEC3B mRNA in leptomeningeal tissue and meningioma tumors. qPCR was used to assess the expression levels of APOBEC3B, which were calculated relative to RPL13A for each specimen and normalized to expression in the adult meninges specimen. The relative APOBEC3B expression in fetal leptomeninges was 4-fold higher than that in normal adult tissue. APOBEC3B expression levels tended to be lower in grade III compared to grade I and II tumors, but were not markedly different (Fig. 2).

Sequencing of APOBEC3B DNA in leptomeningeal tissue and meningioma tumors. No point mutations were detected in APOBEC3B in the 2 WHO grade I, 3 grade II and 3 grade III meningiomas.

Effects of PDGF-BB and cerebrospinal fluid on APOBEC3B protein expression. The 20- and 22-week fetal leptomeningeal cells treated with PDGF-BB or human cerebrospinal fluid demonstrated no changes in APOBEC3B protein expression. Additionally, in the WHO grade I and II meningioma cell cultures, these treatments had no effect (data not shown).

Discussion

APOBEC3B mRNA has been demonstrated to be overexpressed in certain epithelial malignancies, including a high proportion of breast, ovarian and lung carcinomas (5,15,16), and has been shown to promote the development of malignancy (7-10). This is considered to be due to an increased transition from dC to

dT and the resultant increased mutation frequency (5). These mutations were commonly found in oncogenes transcribed in tumor cells (17); for example, in lymphoma cell lines, overexpression of APOBEC3B produced an increase in dC to dT mutations in c-Myc (18). In the present study, it was shown that APOBEC3B was present in all of the fetal leptomeninges, 88% of WHO grade I, 100% of grade II and 83% of grade III meningiomas tested, without differences between grades. PCR revealed no overexpression and sequencing revealed no mutation in APOBEC3B. These findings suggest that, in contrast to several other epithelial malignancies, the overexpression and C>T point mutations in the genome of meningiomas may not be critical to the pathogenesis of meningiomas. Nonetheless, the number of WHO grade III meningiomas available for analysis was limited, and we therefore cannot exclude the possibility that some subtypes of grade III meningiomas may exhibit overexpression.

High APOBEC expression was identified in a few, particularly WHO grade II, meningiomas. Like the majority of meningiomas in this study, these were from tumors in female patients. However, these were distributed amongst histological subtypes with no distinct predilection for site. Nonetheless, the number from any location was limited.

Based on embryological studies, other deaminase DNA-editing enzyme families may be more important in the pathogenesis of meningioma. For example, while the the APOBEC family is important in embryogenesis, the adenosine deaminase acting on RNA (ADAR) family is more important in brain development (19). Nonetheless, the current study identified no definite changes in APOBEC expression among the meningiomas in this series.

References

1. Avesson L and Barry G: The emerging role of RNA and DNA editing in cancer. Biochim Biophys Acta 1845: 308-316, 2014.
2. Kuong KJ and Loeb LA: APOBEC3B mutagenesis in cancer. Nat Genet 45: 964-965, 2013.
3. Benne R, Van den Burg J, Brakenhoff JP, Sloof P, Van Boom JH and Tromp MC: Major transcript of the frameshifted coxII gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. Cell 46: 819-826, 1986.
4. Burns MB, Lackey L, Carpenter MA, Rathore A, Land AM, Leonard B, Refsland EW, Kotandeniya D, Tretyakova N, Nikas JB, *et al*: APOBEC3B is an enzymatic source of mutation in breast cancer. Nature 494: 366-370, 2013.
5. Burns MB, Temiz NA and Harris RS: Evidence for APOBEC3B in multiple human cancers. Nat Genet 45: 977-983, 2013.
6. Lada AG, Dhar A, Boissy RJ, Hirano M, Rubel AA, Rogozin IB and Pavlov YI: AID/APOBEC cytosine deaminase induces genome-wide kataegis. Biol Direct 7: 47, 2012.
7. Taylor BJ, Nik-Zainal S, Wu YL, Stebbings LA, Raine K, Campbell PJ, Rada C, Stratton MR and Neuberger MS: DNA deaminases induce break-associated mutation showers with implication of APOBEC3B and 3A in breast cancer kataegis. ELife 2: e00534, 2013.
8. Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, Kiezun A, Kryukov GV, Carter SL, Saksena G, *et al*: An APOBEC cytadine deaminase pattern is widespread in human cancers. Nat Genet 45: 970-976, 2013.
9. Okuyama S, Marusawa H, Matsumoto T, Ueda Y, Matsumoto Y, Endo Y, Takai A and Chiba T: Excessive activity of Apolipoprotein B mRNA editing enzyme catalytic polypeptide 2 (APOBEC2) contributes to liver and lung tumorigenesis. Int J Cancer 130: 1294-1301, 2012.
10. Jia P, Pao W and Zhao Z: Patterns and processes of somatic mutations in nine major cancers. BMC Med Genomics 7: 11, 2014.
11. Goutagny S, Yang HW, Zuchman-Rossi J, Chan J, Dreyfuss JM, Park PJ, Black PM, Giovanni M, Carroll RS and Kalamarides M: Genomic profiling reveals alternative genetic pathways of meningioma malignant progression dependent on the underlying NF2 status. Clin Cancer Res 16: 4155-4164, 2010.
12. Perry A, Louis DN, Scheithauer BW, Budka H and von Deimling A: Meningiomas. In: Tumours of the Nervous System. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds). WHO Press, Geneva, Switzerland, pp164-172, 2007.
13. Johnson MD, O'Connell M, Facik M, Maurer P, Jahromi B and Pilcher W: Cerebrospinal fluid stimulates leptomeningeal and meningioma cell proliferation and activation of STAT3. J Neurooncol 107: 121-131, 2012.
14. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408, 2001.
15. Leonard B, Hart SN, Burns MB, Carpenter MA, Temiz NA, Rathore A, Vogel RI, Nikas JB, Law EK, Brown WL, *et al*: APOBEC3B upregulation and genomic mutation patterns in serous ovarian carcinoma. Cancer Res 73: 7222-7231, 2013.
16. Sasaki H, Suzuki A, Tatematsu T, Shitara M, Hikosaka Y, Okuda K, Moriyama S, Yano M and Fujii Y: APOBEC3B gene overexpression in non-small-cell lung cancer. Biomed Rep 2: 392-395, 2014.
17. Shinohara M, Io K, Shindo K, Matsui M, Sakamoto T, Tada K, Kobayashi M, Kadowaki N and Takaori-Kondo A: APOBEC3B can impair genomic stability by inducing base substitutions in genomic DNA in human cells. Sci Rep 2: 806, 2012.
18. Rebhandal S, Huemer M, Gassner FJ, Zaborsky N, Hebenstreit D, Catakovic K, Grössinger EM, Greil R and Geisberger R: APOBEC3 signature mutations in in chronic lymphocytic leukemia. Leukemia 28: 1929-1932, 2014.
19. Avesson L and Barry G: The emerging role of RNA and DNA editing in cancer. Biochim and Biophys Acta 1845: 308-316, 2014.