Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

*Review*

# Focusing on the brighter side of Sevoflurane: Realizing true potential of an anesthetic agent as a regulator of cell signaling pathways and microRNAs in different cancers

Muhammad Zahid Qureshi[1], Rukset Attar[2*], Anam Javed[3], Uteuliyev Yerzhan Sabitaliyevich[4], Aima Adylova[4], Konysbayeva Kenzhekul Konysbayevna[5], Aleksandra Buha[6], Muhammad Imran Sohail[7], Aliye Aras[8]

[1] Deanship of Educational services, Department of Biochemistry, Qassim University, Saudi Arabia
[2] Department of Obstetrics and Gynecology, Yeditepe University, Turkey
[3] Wexham Park Hospital, Wexham Street, Slough, Berkshire, SL2 4HL, United Kingdom
[4] Department of Postgraduate Education and Research, Kazakhstan Medical University KSPH, Almaty, Kazakhstan
[5] Ministry of Health of the Republic of Kazakhstan. Zhibek zholy 5, Almaty, Kazakhstan
[6] Department of Toxicology "Akademik Danilo Soldatovic", University of Belgrade-Faculty of Pharmacy, Serbia
[7] Department of Zoology, GCU Lahore, Pakistan
[8] Department of Botany, Faculty of Science, Istanbul University, Istanbul, Turkey

[*]**Correspondence to:** ruksetattar@hotmail.com

**Received** October 23, 2019; **Accepted** November 1, 2019; **Published** December 31, 2019

**Doi:** http://dx.doi.org/10.14715/cmb/2019.65.8.2



**Abstract:** Reconceptualization of different anesthetics as anticancer agents has opened new horizons for a better and sharper analysis of true potential of Sevoflurane as a promising and frontline candidate in the pipeline of anticancer agents. Sevoflurane mediated regulation of cell signaling pathways and non-coding RNAs has leveraged our understanding to another level. There have been remarkable advancements in unraveling mechanistic insights related to the ability of sevoflurane to modulate microRNAs in different cancers. Astonishingly, sevoflurane mediated regulation of miRNAs and long non-coding RNAs have been more comprehensively addressed in ischemia-reperfusion injuries. However, researchers yet have to gather missing pieces of premium research-work to uncover mechanistic regulation of long non-coding RNAs by sevoflurane in various cancers. Sevoflurane modulated control of miRNAs have been reported in glioma, colorectal cancer, breast cancer and hepatocellular carcinoma. In this review we have attempted to summarize most recent cutting edge and high-impact experimental researches which have elucidated myriad of underlying mechanisms modulated by sevoflurane to inhibit cancer development and progression. Despite some of the amazing pharmacological properties of sevoflurane, it has been shown to possess darker side because of its involvement in positive regulation of metastasis. In accordance with this notion we have also summarized how sevoflurane enhanced migratory potential of different cancer cells in a separate section. Therefore, these aspects have to be tested in better designed experimental models to identify most relevant types of cancers which can be therapeutically targeted by sevoflurane.

***Key words:*** Apoptosis; Cancer, Therapy; Sevoflurane; Anesthetics.

## Introduction

Based on the rapidly emerging evidence related to use of volatile anesthetics as modulators of deregulated cell signaling pathways in different cancers, interdisciplinary researchers have witnessed a sharp division of opinion between opponents and proponents. Excitingly, this difference of opinion has impelled researchers to drill down deep into the cellular and molecular biology for re-consideration of volatile anesthetics as anticancer agents. Dissemination of tumor cells during surgery has remained a major stumbling block and one of the major causes of post-operative metastasis. Wealth of information obtained through a critical analysis of the effects exerted by anesthetics at cellular levels has sparked an interest in unraveling the gene regulatory networks modulated by these anesthetics. In this review we have set spotlight on ability of sevoflurane to modulate oncogenic protein network and microRNAs in different cancers.

## Regulation of cell signaling pathways by sevoflurane in different cancers

Deregulation of cell signaling pathways played a central role in cancer development and progression. In this section, we will summarize recent updates related to regulation of different oncogenic proteins and transduction cascades in different cancers by sevoflurane.

Sevoflurane inhibited migration of SCC-4 cells. Sevoflurane attenuated hypoxia-induced stimulation of VEGF in tongue squamous cell carcinoma cells by enhancement of DNA methylation at promoter region of VEGF (1).

Sevoflurane exerted inhibitory effects on platelets activation of patients undergoing lung cancer surgery. GPIIb/IIIa also known as αIIbβ3 and CD62P are present on surface of platelets and have been shown to centrally regulate lung metastasis of cancer cells (2). Levels of CD62P and GPIIb/IIIa were found to be considerably enhanced in platelets activated after surgery. However, sevoflurane significantly reduced the levels of CD62P

and GPIIb/IIIa and remarkably counteracted platelets-induced invasive potential of lung cancer cells (2). Overall these findings clearly suggested that sevoflurane suppressed platelets-mediated increase in invasive capacity of lung cancer cells.

Different clinical researches have shown that regional anesthesia improved post-operative outcomes and reduced the chances of infection by exerting immunostimulatory effects. Liver mononuclear cells (MNCs) include NK cells and natural killer T (NKT) cells which effectively inhibit liver metastasis and have been shown to play critical and instrumental role in inhibiting pulmonary metastases (3). Surgical stress has remained focus of discussion because it repressed cytotoxic activities of liver MNCs and simultaneously enhanced the growth of metastatic liver tumors (3). More importantly, liver MNC function was found to be more sensitive to surgical stress as compared to peripheral blood mononuclear cell function. Spinal block and sevoflurane combinatorially safeguarded the functionality of liver MNCs by maintenance of balance in the T helper 1 (Th1)/ Th2 in response to surgery and reduced metastasis after surgery (3).

Activated Rho proteins bind to and activate ROCK1 and ROCK2 which phosphorylate MYPT1 and MLC. Shown in figure 1. Sevoflurane markedly inhibited phosphorylation of MYPT1 (myosin phosphatase-targeting subunit 1), MLC (myosin light chain), ERK and AKT (4). Overall these findings provided clear proof that sevoflurane not only suppressed RhoA and Ras activities but also downstream signaling pathways centrally involved in uncontrolled proliferation and migration of cervical cancer cells.

Combinatorial targeting of ROCK1 and MEK/ERK pathway has been shown to enhance killing of cancer cells (5,6).

Sevoflurane dose-dependently reduced β-catenin levels and its downstream effectors in CML CD34 and K562 cells (7). Additionally, Wnt/β-catenin-induced transcriptional upregulation of MYC, CYCLIN D1 and BCL9 was also found to be remarkably reduced in K562 cells exposed to sevoflurane. Shown in figure. These findings indicated that sevoflurane inhibited Wnt/β-catenin pathway in CML cells (7).

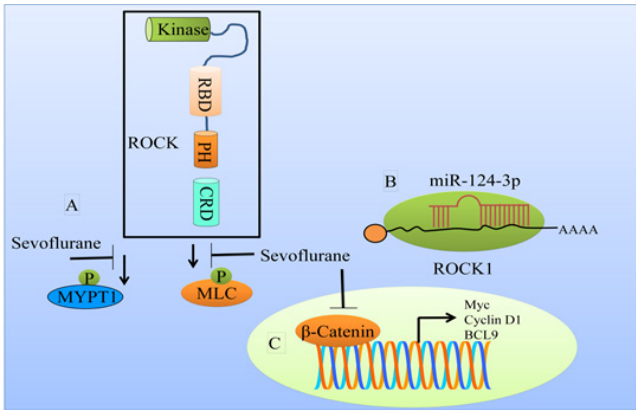


**Figure 1.** (A) ROCK mediated phosphorylation of MYPT1 and MLC. Sevoflurane inhibited phosphorylation of MYPT1 and MLC. (B) ROCK1 is directly targeted by miR-124-3p. (C) Sevoflurane also inhibited β-catenin-mediated transcriptional upregulation of Myc, cyclin D1 and BCL9.

Sevoflurane suppressed hypoxia-induced growth and metastasizing potential of lung cancer cells (8). Likewise, HIF-1α, XIAP and survivin were found to be considerably downregulated in lung cancer cells co-treated with sevoflurane and hypoxic conditions as compared to hypoxia treatment alone. Dimethyloxaloylglycine (DMOG), a HIF-1α agonist severely abolished the inhibitory effects exerted by sevoflurane on hypoxia-induced growth and metastasizing ability of lung cancer cells (8).

Sevoflurane significantly reduced phosphorylated levels of Raf, MEK1/2/ and ERK1/2 in SW480 cells (9). Additionally, sevoflurane reduced MMP2 and MMP9 levels in SW480 cells. Furthermore, sevoflurane inhibited epithelial-to- mesenchymal transition (EMT) mainly through upregulation of E-cadherin and simultaneous downregulation of vimentin and N-cadherin (9).

## Regulation of microRNAs by Sevoflurane in different cancers

Non-coding RNAs have revolutionized the field of molecular oncology [10,11,12]. There has been an explosion in the cancer biology and non-coding RNAs have been shown to modulate wide-ranging oncogenic and tumor suppressor mRNAs[13,14]. In this section, we will discuss how sevoflurane modulated different microRNAs to regulate cancer development and progression.

### Hepatocellular carcinoma

Phosphatase and tensin homolog (PTEN) is a tumor suppressor that classically inhibits the PI3K/AKT/mTOR signaling pathway. Experimental models of PTEN loss have uncovered the molecular mechanisms which drive carcinogenesis. These insights have not only taught us about biological mechanisms but also enabled us to search for strategies to activate PTEN and inhibit PI3K/AKT pathway.

Mechanistically it was shown that sevoflurane enhanced PTEN expression and simultaneously reduced phosphorylated levels of p-PI3K and p-AKT in hepatocellular carcinoma cells (15). Sevoflurane also stimulated the expression of miR-29a in HCC cells. miR-29a directly targeted DNMT3a (DNA methyltransferase 3a) and inhibited PI3K/AKT pathway via activation of PTEN (15).

### Glioma

Sevoflurane treatment inhibited migratory and invasive potential of glioma cells (16). Excitingly, miR-146b-5p was enhanced whereas MMP16 levels were found to be notably reduced in sevoflurane-exposed glioma cells. It has been experimentally verified that overexpression of MMP16 or miR-146b-5p knockdown caused reversal of sevoflurane-induced inhibition of migration and invasion of glioma cells (16).

ROCK1 (Rho-associated, coiled-coil-containing protein kinase-1) is a serine/threonine kinase (17). ROCK1 plays integral role in enhancing migratory and invasive potential of glioma cells. miR-124-3p directly targeted ROCK1. It was shown that miR-124-3p quantitatively controlled the expression of ROCK1 in sevo-

flurane-exposed glioma cells (17).

Phosphorylated AKT levels were found to be drastically reduced in miR-637 overexpressing glioma cells. Sevoflurane stimulated the expression of miR-637 and exerted repressive effects on p-AKT levels (18).

## Colorectal cancer

miR-34a has emerged as a versatile tumor suppressor miRNA having scientifically validated potential to target myriad of oncogenic mRNAs in different cancers. Sevoflurane effectively stimulated the expression of miR-34a in colorectal cancer cells [19]. However, these effects were reversed by pre-treatment with miR-34a inhibitors. ADAM10 (A Disintegrin and metalloproteinase domain-containing protein-10) was critical in various stages of colorectal cancer. ADAM10 was negatively regulated by miR-34a in colorectal cancer cells. ADAM10 overexpression interfered with miR-34a and sevoflurane-induced inhibitory effects on proliferation, migration and invasion abilities of CRC cells (19).

Notch signaling pathway played central role in progression of colorectal cancer (20). Therapeutic targeting of Notch pathway will certainly be helpful in improving drug sensitivity. miR-34a pleiotropically modulated myriad of genes (21). Therefore, ADAM10 inhibition and miR-34a re-expression will inhibit migratory and invasive potential of cancer cells.

Roundabout1 (Robo1) also played central role in progression of colorectal cancer. Sevoflurane potentiated miR-203 mediated targeting of Robo1 and also reduced phosphorylated levels of ERK in CRC cells (22). miR-203 inhibition markedly abolished the inhibitory effects of sevoflurane on phosphorylated ERK levels in CRC cells (22).

Overall these findings provided clear hints about potential role of sevoflurane in regulation of tumor suppressor miRNAs. Future studies will converge on identification of additional tumor suppressor and oncogenic miRNAs which can be effectively modulated by sevoflurane in different cancers.

## Darker side of Sevoflurane

Different volatile anesthetics (isoflurane, sevoflurane, desflurane) have previously been shown to trigger metastasis via upregulation of CXCR2 (C-X-C chemokine receptor type-2) (23). Volatile anesthetics-induced migration was drastically reduced in CXCR2-silenced SKOV3 ovarian cancer cells (23).

Osteopontin (OPN) is a secreted phosphoprotein frequently overexpressed in many cancers. Sevoflurane was found to enhance migratory potential of non-small cell lung cancer cells (A549) and renal cell carcinoma (RCC4) [24]. Sevoflurane upregulated TGFβ1, TGFβRII and OPN but reduced nuclear SMAD3 in cisplatin-treated RCC4 cells. However, surprisingly sevoflurane potentiated migratory ability of A549 cells by increment of nuclear SMAD3 levels (24).

LFA-1 (Leukocyte function-associated antigen-1) is an essential adhesion molecule on NK cells25. Sevoflurane and Isoflurane abrogated the conjugation of K562 cells with NK92-MI cells. Sevoflurane and Isoflurane attenuated tumor cytotoxicity of natural killer cells partially through inhibition of LFA-1 (25).

Sevoflurane time- and dose- dependently upregulated HIF-1α, HIF-2α, VEGF and p-AKT in glioma stem cells (26). RNA interference strategy against HIFs considerably reduced the percentage of proliferating GSCs after exposure of sevoflurane. Similarly, pre-treatment with AKT inhibitor also interfered with sevoflurane-mediated upregulation of HIFs (26). Collectively these findings suggested that sevoflurane potentiated the proliferation of GSCs through HIFs driven pathway.

Calpains belong to the family of cysteine proteases that catalyze the proteolysis of a large number of specific substrates. Sevoflurane exposure concentration-dependently enhanced the activity of calpains and CD44 protein in A172 and U251 cells (27). CD44 Knockdown with siRNA abrogated sevoflurane-induced increase in calpain activity, migratory, invasive and colony-forming capacity of U251 cells. It was observed that inhalation of 4% sevoflurane induced significant increase in the tumor volume, migration and invasion in nude mice xenografted with glioblastoma U87 cells (27).

## Concluding remarks

Series of research reports have started to highlight preventive/inhibitory role of different anesthetics against cancer. Sevoflurane has gradually gained attention because of its promising ability to inhibit cancer progression. However, despite the initial promising results obtained in cancer cell lines, sevoflurane has to pass touchstone tests in molecular oncology. There are visible knowledge gaps in our understanding about ability of sevoflurane to modulate oncogenic cell signaling pathways.

It has recently been revealed that sevoflurane and propofol differentially influenced miRNAs in circulating extracellular vesicles during colorectal cancer resection (28). Sevoflurane has been shown to modulate various sets of miRNAs in different cancers but these findings need additional support by exposure of tumor bearing mice to sevoflurane.

## References

1. Lu Y, Wang J, Yan J, Yang Y, Sun Y, Huang Y, Hu R, Zhang Y, Jiang H. Sevoflurane attenuate hypoxia-induced VEGF level in tongue squamous cell carcinoma cell by upregulating the DNA methylation states of the promoter region. Biomed Pharmacother. 2015;71:139-45.

2. Liang H, Yang CX, Zhang B, Zhao ZL, Zhong JY, Wen XJ. Sevoflurane attenuates platelets activation of patients undergoing lung cancer surgery and suppresses platelets-induced invasion of lung cancer cells. J Clin Anesth. 2016 ;35:304-312.

3. Wada H, Seki S, Takahashi T, Kawarabayashi N, Higuchi H, Habu Y, Sugahara S, Kazama T. Combined spinal and general anesthesia attenuates liver metastasis by preserving TH1/TH2 cytokine balance. Anesthesiology. 2007;106(3):499-506.

4. Ding J, Zhang L, Zeng S, Feng T. Clinically relevant concentration of sevoflurane suppresses cervical cancer growth and migration through targeting multiple oncogenic pathways. Biochem Biophys Res Commun. 2019 ;514(4):1179-1184.

5. Vogel CJ, Smit MA, Maddalo G, Possik PA, Sparidans RW, van der Burg SH, Verdegaal EM, Heck AJ, Samatar AA, Beijnen JH, Altelaar AF, Peeper DS. Cooperative induction of apoptosis in NRAS

mutant melanoma by inhibition of MEK and ROCK. Pigment Cell Melanoma Res. 2015;28(3):307-17.
6. Nakabayashi H, Shimizu K. HA1077, a Rho kinase inhibitor, suppresses glioma-induced angiogenesis by targeting the Rho-ROCK and the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) signal pathways. Cancer Sci. 2011;102(2):393-9.
7. Ruan X, Jiang W, Cheng P, Huang L, Li X, He Y, Mai M, Tan Z. Volatile anesthetics sevoflurane targets leukemia stem/progenitor cells via Wnt/β-catenin inhibition. Biomed Pharmacother. 2018;107:1294-1301.
8. Liang H, Yang CX, Zhang B, Wang HB, Liu HZ, Lai XH, Liao MJ, Zhang T. Sevoflurane suppresses hypoxia-induced growth and metastasis of lung cancer cells via inhibiting hypoxia-inducible factor-1α. J Anesth. 2015;29(6):821-30.
9. Yang X, Zheng YT, Rong W. Sevoflurane induces apoptosis and inhibits the growth and motility of colon cancer in vitro and in vivo via inactivating Ras/Raf/MEK/ERK signaling. Life Sci. 2019:116916.
10. Farooqi AA, Fayyaz S, Shatynska-Mytsyk I, Javed Z, Jabeen S, Yaylim I, Gasparri ML, Panici PB. Is miR-34a a Well-equipped Swordsman to Conquer Temple of Molecular Oncology? Chem Biol Drug Des. 2016;87(3):321-34.
11. Farooqi AA, Qureshi MZ, Coskunpinar E, Naqvi SK, Yaylim I, Ismail M. MiR-421, miR-155 and miR-650: emerging trends of regulation of cancer and apoptosis. Asian Pac J Cancer Prev. 2014;15(5):1909-12.
12. Mytsyk Y, Dosenko V, Borys Y, Kucher A, Gazdikova K, Busselberg D, Caprnda M, Kruzliak P, Farooqi AA, Lubov M. MicroRNA-15a expression measured in urine samples as a potential biomarker of renal cell carcinoma. Int Urol Nephrol. 2018;50(5):851-859.
13. Wen R, Umeano AC, Essegian DJ, Sabitaliyevich UY, Wang K, Farooqi AA. Role of microRNA-410 in molecular oncology: A double edged sword. J Cell Biochem. 2018;119(11):8737-8742.
14. Gasparri ML, Besharat ZM, Farooqi AA, Khalid S, Taghavi K, Besharat RA, Sabato C, Papadia A, Panici PB, Mueller MD, Ferretti E. MiRNAs and their interplay with PI3K/AKT/mTOR pathway in ovarian cancer cells: a potential role in platinum resistance. J Cancer Res Clin Oncol. 2018;144(12):2313-2318.
15. Song G, Tian L, Cheng Y, Liu J, Wang K, Li S, Li T. Antitumor activity of sevoflurane in HCC cell line is mediated by miR-29a-induced suppression of Dnmt3a. J Cell Biochem. 2019;120(10):18152-18161.
16. Zhang L, Wang J, Fu Z, Ai Y, Li Y, Wang Y, Wang Y. Sevoflurane suppresses migration and invasion of glioma cells by regulating miR-146b-5p and MMP16. Artif Cells Nanomed Biotechnol. 2019;47(1):3306-3314.
17. Gao C, Shen J, Meng ZX, He XF. Sevoflurane Inhibits Glioma Cells Proliferation and Metastasis through miRNA-124-3p/ROCK1 Axis. Pathol Oncol Res. 2019 Mar 26.
18. Yi W, Li D, Guo Y, Zhang Y, Huang B, Li X. Sevoflurane inhibits the migration and invasion of glioma cells by upregulating microRNA-637. Int J Mol Med. 2016;38(6):1857-1863.
19. Sun SQ, Ren LJ, Liu J, Wang P, Shan SM. Sevoflurane inhibits migration and invasion of colorectal cancer cells by regulating microRNA-34a/ADAM10 axis. Neoplasma. 2019. pii: 181213N962.
20. Farooqi AA, de la Roche M, Djamgoz MBA, Siddik ZH. Overview of the oncogenic signaling pathways in colorectal cancer: Mechanistic insights. Semin Cancer Biol. 2019;58:65-79.
21. Farooqi AA, Tabassum S, Ahmad A. MicroRNA-34a: A Versatile Regulator of Myriads of Targets in Different Cancers. Int J Mol Sci. 2017 ;18(10).
22. Fan L, Wu Y, Wang J, He J, Han X. Sevoflurane inhibits the migration and invasion of colorectal cancer cells through regulating ERK/MMP-9 pathway by up-regulating miR-203. Eur J Pharmacol. 2019 ;850:43-52.
23. Iwasaki M, Zhao H, Jaffer T, Unwith S, Benzonana L, Lian Q, Sakamoto A, Ma D. Volatile anaesthetics enhance the metastasis related cellular signalling including CXCR2 of ovarian cancer cells. Oncotarget. 2016;7(18):26042-56.
24. Ciechanowicz S, Zhao H, Chen Q, Cui J, Mi E, Mi E, Lian Q, Ma D. Differential effects of sevoflurane on the metastatic potential and chemosensitivity of non-small-cell lung adenocarcinoma and renal cell carcinoma in vitro. Br J Anaesth. 2018;120(2):368-375.
25. Tazawa K, Koutsogiannaki S, Chamberlain M, Yuki K. The effect of different anesthetics on tumor cytotoxicity by natural killer cells. Toxicol Lett. 2017 ;266:23-31.
26. Shi QY, Zhang SJ, Liu L, Chen QS, Yu LN, Zhang FJ, Yan M. Sevoflurane promotes the expansion of glioma stem cells through activation of hypoxia-inducible factors in vitro. Br J Anaesth. 2015;114(5):825-30.
27. Lai RC, Shan WR, Zhou D, Zeng XQ, Zuo K, Pan DF, Zeng WA, Zuo ZY. Sevoflurane promotes migration, invasion, and colony-forming ability of human glioblastoma cells possibly via increasing the expression of cell surface protein 44. Acta Pharmacol Sin. 2019. doi: 10.1038/s41401-019-0221-0.
28. Buschmann D, Brandes F, Lindemann A, Maerte M, Ganschow P, Chouker A, Schelling G, Pfaffl MW, Reithmair M. Propofol and Sevoflurane Differentially Impact MicroRNAs in Circulating Extracellular Vesicles during Colorectal Cancer Resection: A Pilot Study. Anesthesiology. 2019 Oct 4. doi: 10.1097/ALN.0000000000002986.