The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2020

Volume 10, Pages 29-36

ICVALS 2020: International Conference on Veterinary, Agriculture and Life Sciences

Evaluation of the Effect of RhoB Inhibition on Cancer Stem Cell Properties in NSCLC A549 Cells

Fathiya RAHMI
Gaziantep University

Hiba KHAIR
Gaziantep University

Isık Didem KARAGOZ
Gaziantep University

Abstract: The cancer stem cells model suggests that a small subgroup of cancer cells can self-renew and substantially leads to the recurrence of tumors. Such cells are thought to be a reservoir for the regeneration of tumor-initiating mutant cells, which are insensitive to the chemotherapy currently being used. RhoB GTPase is implicated in regulating cell survival, tumorigenesis, angiogenesis, migration, and metastasis. Higher degrees of tumor progression and invasiveness are associated with RhoB expression. The contribution of Rho GTPases, in particular RhoB, in cancer stem cell properties, has not yet been fully explored. This study aims to explore the possible relationship between RhoB expression and the in vitro cancer stem cell-like characteristics of non-small cell lung cancer cells such as spheroid formation and self-renewal. To determine whether the depletion of RhoB could promote CSC-like characteristics, RhoB siRNA or AllStars siRNA negative control were transfected into A549 cells. The spheroid formation assay was used to evaluate the ability of cells to grow in anchorage-independent conditions to form spheroids. The results demonstrated that RhoB knockdown did not have a statistically significant effect in promoting cancer stem-like properties in A549 cells. This study may lead to further understanding of the contribution of RhoB to in vitro cancer stem cell features such as, the formation of tumorspheroids.

Keywords: CSCs, RhoB siRNA, Spheroid formation, Self-renewal.

Introduction

Lung cancer is the main cause of cancer deaths worldwide with both high mortality and morbidity rates due to the limited therapeutic options (Kalemkerian et al., 2013; Siegel et al., 2016). Approximately 80% of lung cancers are non-small cell lung cancers, which are non-neuroendocrine tumors including adenocarcinomas, large cell carcinoma and squamous cell carcinoma (Parsons et al., 2010; Siegel et al., 2016). Even with the most advanced imaging, staging, surgical strategies, chemotherapy, radiotherapy, as well as personalized treatments with targeted therapies such as EGFR tyrosine kinase inhibitors and ALK inhibitors in specific patient groups, the five-year survival rate for patients is only 5–15% (Kato et al., 2004; Winton et al., 2005; Leon et al.,2016).

The stem cell model of carcinogenesis suggests that cancers arise from cells with stem-like characteristics as a result of the impaired regulation of self-renewal pathways. Such dysregulation stimulates the expansion of this cell population that then may undergo additional genetic or epigenetic modifications to turn into a fully transformed clone (Wicha et al., 2006).

Tumor heterogeneity and the presence of small subsets of cells with stem-like properties called cancer stem cells (CSCs) have been reported in almost all malignancies during the past decade (Leon et al.,2016). CSCs typically exhibit many features of embryonic or tissue stem cells including slow growth rates and insensitivity to chemotherapy and/or radiation therapy, therefore new treatment modalities that selectively target these cells are needed to control stem cell survival, proliferation, and differentiation (Jordan et al., 2006).

Increasing evidence proposes that lung cancer incorporates a cancer stem cell subpopulation that is responsible for the onset, spread, and metastasis of the tumor (Ho et al.,2007; Eramo et al.,2008; Levina et al.,2008; Bertolini et al.,2009; Jiang et al.,2009, O'Flaherty et al.,2012; Wang et al.,2013). Since CSCs cells have been implicated in tumorigenicity, cancer progression, and therapeutic resistance of lung cancer, their study would provide important insights into the understanding of their biology, expansion, and maintenance of, as well as to the development of novel diagnostic and prognostic tests and exploring novel therapeutic targets (Pine et al.,2008; Peacock and Watkins ,2008; Miyata et al.,2015; Zakaria et al.,2017). However, each type of tumor comprises different progenitor or stem cell types that are controlled by various molecular pathways, which results in variation in the expression of markers in lung cancer subtypes making these cells more difficult to be identified and targeted (Zakaria et al.,2017).

The propensity to self-renew and produce differentiated progeny constitutes the basic features of cancer stem cells (Ponti et al., 2005; Eramo et al., 2008). Tumorspheroids are a typical descriptor of CSCs that can maintain stemness profiles in proliferation, differentiation and under serum-free culturing conditions (Weiswald et al., 2015). Sphere formation assay has been widely used as a surrogate assay for the enrichment of CSCs or cancer cells with stem-like characteristics from solid tumors. Tumorspheroids culture systems are based on the ability of stem/progenitor cells to survive and form floating 3-dimensional spheroid bodies in semisolid media, such as collagen or Matrigel or in low attachment plates, in serum-free growth medium containing epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) (Ishiguro et al.,2017). Spheroid cultures as a method of enrichment for cancer stem cells have been used in many human cancers and cancer cell lines including glioma, melanoma, sarcoma, lung cancer, renal cancer, and rhabdomyosarcoma (Singh et al., 2003; Fang et al., 2005; Gibbs et al., 2005; Eramo et al., 2008; Zhong et al.,2010; Walter et al., 2011).

RhoA, RhoB, and RhoC are a subgroup of GTPases belonging to the family of GTP binding proteins involved in the regulation of various cellular processes, including cytoskeletal regulation, cell morphology, adhesion, motility, cell survival, cell cycle progression and gene expression (Bishop and Hall, 2000; Bar-Sagi and Hall, 2000; Aznar and Lacal, 2001; Sahai and Marshall, 2002; Calvayrac et al., 2016). RhoB is associated with cell survival, migration, angiogenesis, tumor formation, and metastasis (Huang and Prendergast, 2006; Karlsson et al., 2009; Ridley, 2013). Several factors, including growth factors such as PDGF, EGF, and TGF-β, UV radiation, and DNA damaging substances, can induce RhoB expression (Jähner and Hunter, 1991; De Cremoux et al., 1994; Fritz et al., 1995; Engel et al., 1998).

Downregulation of RhoB has been associated with higher degrees of tumor progression and invasiveness, ranging from reduced expression in superficially invasive carcinoma to near disappearance of RhoB expression in deeply infiltrating carcinoma (Adnane et al., 2002). Several studies have reported that lung cancer progression is accompanied by a lack of RhoB expression (Wang et al., 2003; Mazieres et al., 2004; Sato et al., 2007) and plays an important role in the acquisition of a more aggressive adenocarcinoma phenotype. Based on this, it can be used as a prognostic factor in NSCLC (Calvayrac et al., 2014). Furthermore, RhoB loss has been found to increase the migration and invasion of bronchial cells in vitro and in vivo by a mechanism related to AKT (Bousquet et al., 2009; Bousquet et al., 2016).

In view of all that has been mentioned so far, this study aims to assess cancer stem-like properties in A549 and investigate the contribution of RhoB expression to the in vitro cancer stem cell-like characteristics such as spheroid formation.

Materials and Methods:

Cell Culture:

A549 cells was grown in RPMI-1640 medium supplemented with 10% FBS, 1% L-glutamine and 1% penicillin/streptomycin at 37°C and 5% CO_2 in a humidified incubator. Cells were seeded in 25 cm^2 tissue culture flasks and passaged when 80% confluence was reached.

Transfection with RhoB siRNA

Before transfection, 6×10^4 cells were seeded in 24-well plates in an appropriate culture medium containing serum and antibiotics. For the short time until transfection, the cells were incubated under normal growth conditions (typically 37°C and 5% CO_2). RhoB siRNA or AllStars siRNA negative control (used as RhoB siRNA negative control) was diluted in Opti-MEM I medium without serum. To dilute HiPerfect, Opti-MEM I was added and gently mixed by inverting the tube 2-3 times. Diluted HiPerFect Transfection Reagent was added to the diluted RhoB siRNA and mixed by vortexing. RhoB siRNA or RhoB siRNA negative control (Qiagen) were allowed to form transfection complexes with HiperFect transfection reagent (Qiagen) for 20 minutes at room temperature (15−25°C) in serum-free Opti-MEM I (Invitrogen) at 25 nM final concentration according to the manufacturer's instructions. Then, transfection complexes were added drop-wise onto A549 cells. The plate was swirled gently to ensure uniform distribution of the transfection complexes. The cells were incubated for 24 hours with the transfection complexes under their normal growth conditions. RhoB knockdown was monitored 24 hours after transfection. Transfected cells were harvested for total RNA isolation. The relative expression of RhoB was evaluated by qRT-PCR.

Tumorspheroids Formation Assay:

A549 cells transfected with RHoB siRNA, RhoB siRNA negative control, and untransfected cells were plated at 1000 cells per well in ultra-low attachment 96-well plates (Corning, New York, USA) in spheroid culture medium containing serum-free medium DMEM-F12K (1:1) supplemented with 20 ng/ml EGF, 10 ng/ml bFGF, 1x B27 supplement, 1x insulin, 0.4% Bovine Serum Albumin and 1 % PenStrep. To prevent cell aggregation, methylcellulose was added at a concentration of 1.5 % to the culture medium. The cells were incubated at 37°C for 10 days. At the end of the incubation period; spheroids were counted using an inverted microscope. The diameters of the spheres were analyzed using the Image J-NIH (U. S. National Institutes of Health, Bethesda, MA, USA).

RT-PCR:

Total RNA was isolated from cells using the miRNeasy Kit (Qiagen) according to the manufacturer's protocol. Reverse transscription reaction was carried out using the High-Capacity RNA to cDNA kit (Applied Biosystems). SYBR green-based real time RT-PCR was performed using the Power SYBR Green PCR Master Mix kit (Applied Biosystems) to measure the expression of RhoB in cells by the StepOnePlusTM Real-Time PCR System (Applied Biosystems). GAPDH was used as endogenous control.

Statistical Analysis:

All data are expressed as mean ± SD. Student's *t* test for the comparison between groups was performed using GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, California USA). Those with a *P* value equal 0.05 or less were considered statistically significant.

Results

RT-PCR:

To explore whether RhoB siRNA can decrease the expression of RhoB, A549 cells were transfected with RhoB siRNA or RhoB siRNA negative control (AllStars siRNA negative control) at a final concentration of 25 nM for 24 h, then the relative expression of RhoB was measured by qRT-PCR. The relative expression of RhoB was 0.12 ± 0.01 in A549 cells transfected with RhoB siRNA, which was significantly decreased, as compared to 1.00 ± 0.00 in A549 cells transfected with RhoB siRNA negative control cell group ($p< 0.001$; Fig.1), when analyzed by RT-PCR. The results suggestes that RhoB siRNA could decrease RhoB expression in A549 cells.

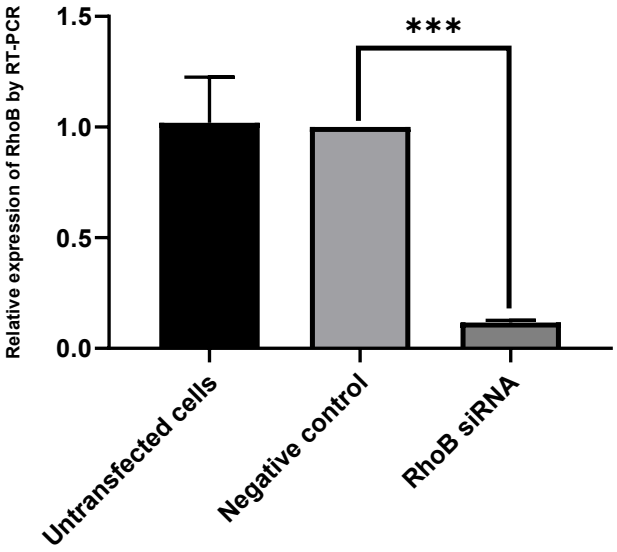

Figure 1. Relative expression of RhoB siRNA by RT-PCR. (*** indicates $p<0.001$).

Tumorsphere Formation Assay Results:

To explore the effects of RhoB siRNA on the growth potential of floating spheroids of A549 stem-like cells, tumorspheroids formation assay was performed. A549 cells were seeded at a density of 1000 cells/well in a total of 24 wells from an ultra-low adherent 96 well plates to obtain spheroids. The medium was changed four times a week during the 10 days incubation period. After 10 days of culture, ball-like spheres were observed. Images of tumor spheroids derived from A549 cells transfected with RhoB SiRNA (Fig. 2A), negative control (Fig. 2B) and untransfected cells (Fig. 2C), respectively were taken with an inverted microscope using a 10x objective lens to observe and analyze spheroids. The spheroid analysis was performed using image processing algorithms of the ImageJ-NIH software program. Spheroids with diameters > 40µm were counted from a total of 10 wells per cel group. The percentage of the spheroid formation efficiency (SFE%) was calculated by dividing the total number of formed spheroids by the total number of seeded living cells and multiplying by 100.

The sphere forming efficiency of A549 transfected with RhoB siRNA (2.20 ± 0.92%; Fig. 3A) was statistically insignificent ($p > 0.05$; Fig. 3A) as compared to A549 cells transfected with negative control (1.78 ± 0.89 %; Fig. 3A) and untransfected cells (2.44 ± 0.85 %; Fig. 3A).

The mean diameter of spheroids formed by A549 cells transfected with RhoB siRNA (52.28 ± 18.74 µm; Table 1; Fig.3B) was statistically insignificent ($p > 0.05$; Table 1; Fig. 3B) as compared to A549 cells transfected with negative control (52.35 ± 10.48 µm; Table 1; Fig.3B) and untransfected cells (52.76 ± 11.00 µm; Table 1; Fig.3B).

Table 1. The diameters of spheroids formed by A549 cells transfected with RhoB siRNA, negative control and untransfected cells.

	A549 cells transfected with RhoB siRNA	A549 cells transfected with negative control	Untransfected cells
Diameter Mean (µm)	52.28 ± 18.74	52.35 ± 10.48	52.76 ± 11.00
P value	0.96		

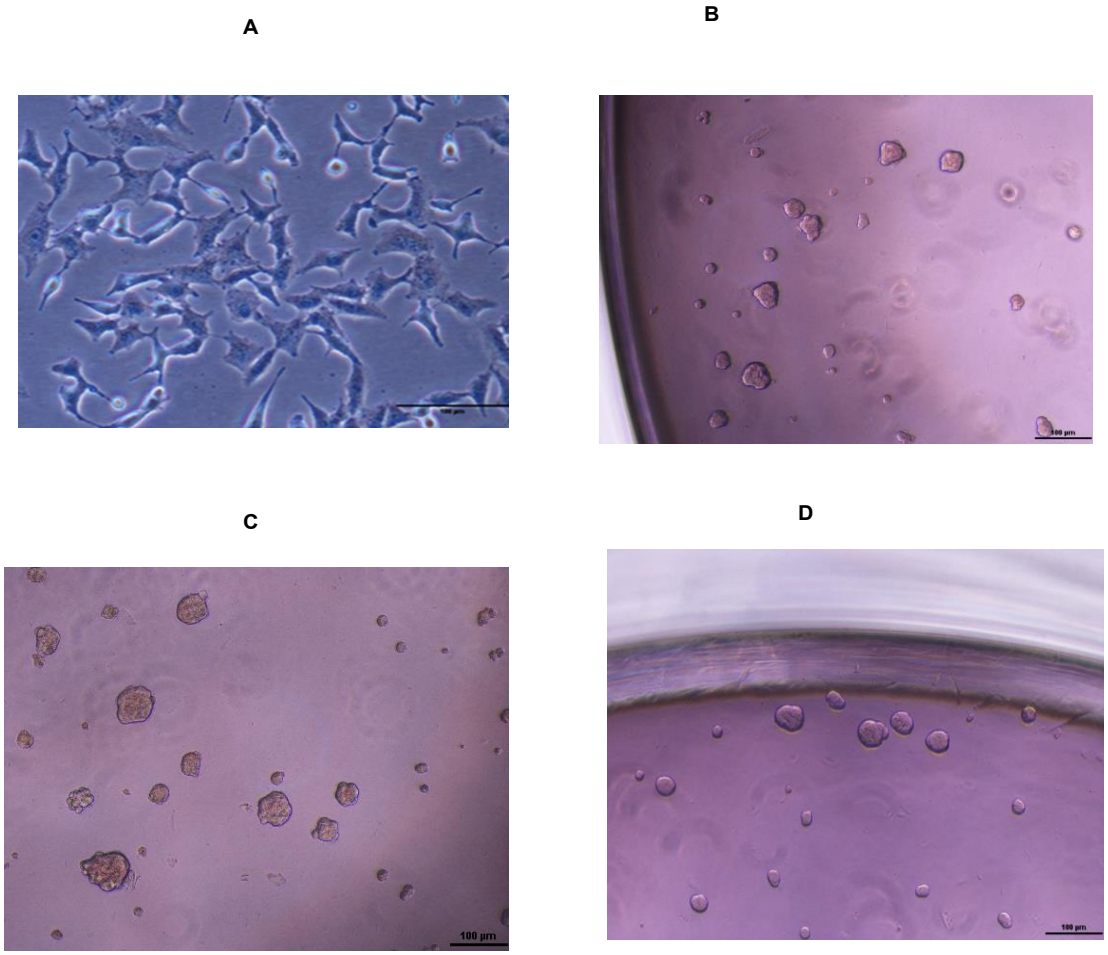

Figure 2. (A) A549 adherent monolayer parental cells. Scale bar is 100µm. Original magnification 20x for A and 10x for (B), (C) and (D). (B), (C) and (D) Tumor spheroids derived from A549 cells transfected with RhoB SiRNA, negative control and untransfected cells, respectively.

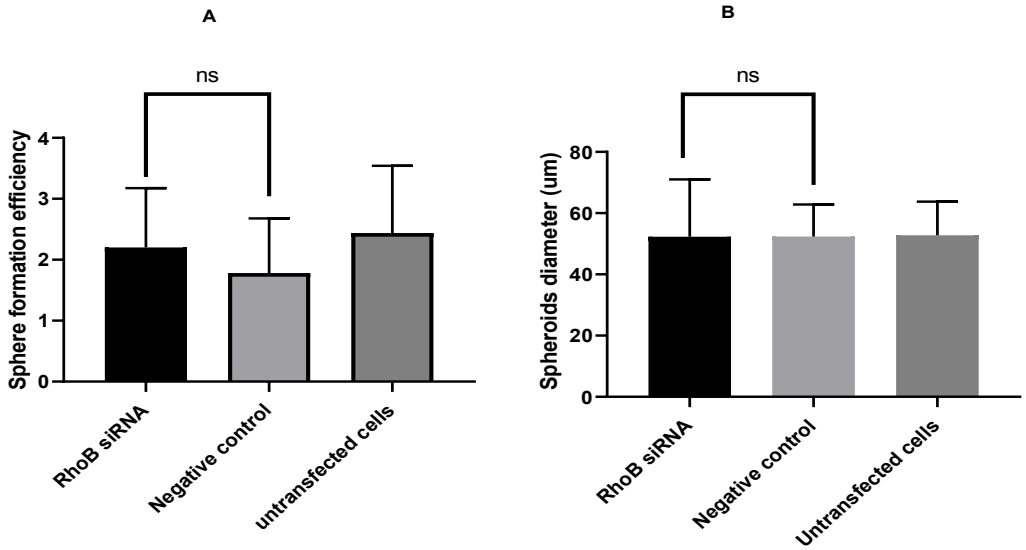

Figure 3. (A) Sphere-forming efficiency of A549 transfected with RhoB siRNA, negative control, and untransfected cells. (ns indicates non-significance, $p > 0.05$). (B) The diameters of spheroids formed by A549 cells transfected with RhoB siRNA, negative control, and untransfected cells. (ns indicates non-significance, $p > 0.05$).

Discussion

Increasing evidence has shown that NSCLC enhanced tumorigenicity, as in other solid cancer types, is driven by a tumor cell population that exhibits stem cell-like characteristics such as self-renewal, differentiation, cell mobility and therapy resistance, and referred to as cancer stem cells (CSC) or tumor-initiating cells (TIC) (Eramo et al.,2008; Bertolini et al.,2009; Magee et al.,2012;Visvader et al.,2012). Further investigation of the CSCs population would provide a deeper understanding of the regulatory mechanisms involved in their maintenance and facilitate the development of therapeutic strategies to control lung cancer in the long term (Liu et al.,2013). To study cancer stem-like properities in NSCLC A549 cells, we used the sphere formation assay, which is a marker-independent approach.

Loss of RhoB expression has been detected in numerous lung cancer cell lines and tumor tissues (Wang et al.,2003; Mazieres et al.,2004; Sato et .,2007; Mazières et al.,2007). In this study, we aimed to assess the effects of RhoB knockdown on cancer stem-like properties, such as tumorspheres formation in A549 NSCLC cells. The inhibition of RhoB did not enhance the capability of A549 cells to form spheroids, suggesting that RhoB loss in cancer cells might be required for other aspects of malignancy, such as acquiring of a motile and invasive phenotype, but not cancer stem-like properties, such as tumorspheres formation.

Recommendations

Recently the cancer stem cells are implicated in tumor initiation, progression, invasion, metastasis, relapse and resistance to chemotherapy and radiotherapy. Since the resistance to conventional chemotherapeutic agents is becoming a growing phenomenon, there is an urgent need to develop novel drugs that specifically target the cancer stem cell populations in tumors. Thus, it is of great value to conduct future studies to broaden our current knowledge of the biology of cancer stem cells and the underlying molecular regulatory mechanisms of their behavior.

References

Adnane, J., Seijo, E., Chen, Z., Bizouarn, F., Leal, M., Sebti, S. M., & Muñoz-Antonia, T. (2002). RhoB, not RhoA, represses the transcription of the transforming growth factor β type II receptor by a mechanism involving activator protein 1. *Journal of Biological Chemistry*, *277*(10), 8500-8507.

Aznar, S., & Lacal, J. C. (2001). Rho signals to cell growth and apoptosis. *Cancer letters*, *165*(1), 1-10.

Bar-Sagi, D., & Hall, A. (2000). Ras and Rho GTPases: a family reunion. *Cell*, *103*(2), 227-238.

Bertolini, G., Roz, L., Perego, P., Tortoreto, M., Fontanella, E., Gatti, L., ... & Roz, E. (2009). Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. *Proceedings of the National Academy of Sciences*, *106*(38), 16281-16286.

Bishop, A. L., & Hall, A. (2000). Rho GTPases and their effector proteins. *Biochemical Journal*, *348*(2), 241-255.

Bousquet, E., Mazières, J., Privat, M., Rizzati, V., Casanova, A., Ledoux, A., ... & Pradines, A. (2009). Loss of RhoB expression promotes migration and invasion of human bronchial cells via activation of AKT1. *Cancer research*, *69*(15), 6092-6099.

Bousquet, E., Calvayrac, O., Mazieres, J., Lajoie-Mazenc, I., Boubekeur, N., Favre, G., & Pradines, A. (2016). RhoB loss induces Rac1-dependent mesenchymal cell invasion in lung cells through PP2A inhibition. *Oncogene*, *35*(14), 1760-1769.

Calvayrac, O., Pradines, A., Raymond-Letron, I., Rouquette, I., Bousquet, E., Lauwers-Cances, V., ... & Milia, J. (2014). RhoB determines tumor aggressiveness in a murine EGFRL858R-induced adenocarcinoma model and is a potential prognostic biomarker for lepidic lung cancer. *Clinical Cancer Research*, *20*(24), 6541-6550.

Calvayrac, O., Mazières, J., Figarol, S., Marty- Detraves, C., Raymond- Letron, I., Bousquet, E., ... & Guibert, N. (2017). The RAS- related GTP ase RHOB confers resistance to EGFR- tyrosine kinase inhibitors in non- small- cell lung cancer via an AKT- dependent mechanism. *EMBO molecular medicine*, *9*(2), 238-250.

De Cremoux, P., Gauville, C., Closson, V., Linares, G., Calvo, F., Tavitian, A., & Olofsson, B. (1994). EGF modulation of the ras- related rhoB gene expression in human breast- cancer cell lines. *International journal of cancer*, *59*(3), 408-415.

Engel, M. E., Datta, P. K., & Moses, H. L. (1998). Signal transduction by transforming growth factor- β: A cooperative paradigm with extensive negative regulation. *Journal of Cellular Biochemistry*, *72*(S30–31), 111-122.

Eramo, A., Lotti, F., Sette, G., Pilozzi, E., Biffoni, M., Di Virgilio, A., ... & De Maria, R. (2008). Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell death and differentiation*, *15*(3), 504.

Fang, D., Nguyen, T. K., Leishear, K., Finko, R., Kulp, A. N., Hotz, S., ... & Herlyn, M. (2005). A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer research*, *65*(20), 9328-9337.

Fritz, G., Kaina, B., & Aktories, K. (1995). The ras-related small GTP-binding protein RhoB is immediate-early inducible by DNA damaging treatments. *Journal of Biological Chemistry*, *270*(42), 25172-25177.

Gibbs, C. P., Kukekov, V. G., Reith, J. D., Tchigrinova, O., Suslov, O. N., Scott, E. W., ... & Steindler, D. A. (2005). Stem-like cells in bone sarcomas: implications for tumorigenesis. *Neoplasia*, *7*(11), 967-976.

Ho, M. M., Ng, A. V., Lam, S., & Hung, J. Y. (2007). Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. *Cancer research*, *67*(10), 4827-4833.

Huang, M., & Prendergast, G. C. (2006). RhoB in cancer suppression. *Histology and histopathology*.

Ishiguro, T., Ohata, H., Sato, A., Yamawaki, K., Enomoto, T., & Okamoto, K. (2017). Tumor- derived spheroids: relevance to cancer stem cells and clinical applications. *Cancer science*, *108*(3), 283-289.

Jähner, D., & Hunter, T. O. N. Y. (1991). The ras-related gene rhoB is an immediate-early gene inducible by v-Fps, epidermal growth factor, and platelet-derived growth factor in rat fibroblasts. *Molecular and Cellular Biology*, *11*(7), 3682-3690.

Jiang, F., Qiu, Q., Khanna, A., Todd, N. W., Deepak, J., Xing, L., ... & Katz, R. L. (2009). Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. *Molecular cancer research*, *7*(3), 330-338.

Jordan, C. T., Guzman, M. L., & Noble, M. (2006). Cancer stem cells. *New England Journal of Medicine*, *355*(12), 1253-1261.

Kalemkerian, G. P., Akerley, W., Bogner, P., Borghaei, H., Chow, L. Q., Downey, R. J., ... & Hayman, J. (2013). Small cell lung cancer. *Journal of the National Comprehensive Cancer Network*, *11*(1), 78-98.

Karlsson, R., Pedersen, E. D., Wang, Z., & Brakebusch, C. (2009). Rho GTPase function in tumorigenesis. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1796*(2), 91-98.

Kato, H., Ichinose, Y., Ohta, M., Hata, E., Tsubota, N., Tada, H., ... & Ohta, M. (2004). A randomized trial of adjuvant chemotherapy with uracil–tegafur for adenocarcinoma of the lung. *New England Journal of Medicine*, *350*(17), 1713-1721.

Leon, G., MacDonagh, L., Finn, S. P., Cuffe, S., & Barr, M. P. (2016). Cancer stem cells in drug resistant lung cancer: Targeting cell surface markers and signaling pathways. *Pharmacology & therapeutics*, *158*, 71-90.

Levina, V., Marrangoni, A. M., DeMarco, R., Gorelik, E., & Lokshin, A. E. (2008). Drug-selected human lung cancer stem cells: cytokine network, tumorigenic and metastatic properties. *PloS one*, *3*(8), e3077.

Liu, J., Xiao, Z., Wong, S. K. M., Tin, V. P. C., Ho, K. Y., Wang, J., ... & Wong, M. P. (2013). Lung cancer tumorigenicity and drug resistance are maintained through ALDHhiCD44hi tumor initiating cells. *Oncotarget*, *4*(10), 1698.

Magee, J. A., Piskounova, E., & Morrison, S. J. (2012). Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer cell*, *21*(3), 283-296.

Mazieres, J., Antonia, T., Daste, G., Muro-Cacho, C., Berchery, D., Tillement, V., ... & Favre, G. (2004). Loss of RhoB expression in human lung cancer progression. *Clinical cancer research*, *10*(8), 2742-2750.

Mazières, J., Tovar, D., He, B., Nieto-Acosta, J., Marty-Detraves, C., Clanet, C., ... & Favre, G. (2007). Epigenetic regulation of RhoB loss of expression in lung cancer. *BMC cancer*, *7*(1), 220.

Miyata, T., Yoshimatsu, T., So, T., Oyama, T., Uramoto, H., Osaki, T., ... & Gotoh, A. (2015). Cancer stem cell markers in lung cancer. *Personalized Medicine Universe*, *4*, 40-45.

O'Flaherty, J. D., Barr, M., Fennell, D., Richard, D., Reynolds, J., O'Leary, J., & O'Byrne, K. (2012). The cancer stem-cell hypothesis: its emerging role in lung cancer biology and its relevance for future therapy. *Journal of thoracic oncology*, *7*(12), 1880-1890.

Parsons, A., Daley, A., Begh, R., & Aveyard, P. (2010). Influence of smoking cessation after diagnosis of early stage lung cancer on prognosis: systematic review of observational studies with meta-analysis. *Bmj*, *340*, b5569.

Peacock, C. D., & Watkins, D. N. (2008). Cancer stem cells and the ontogeny of lung cancer. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*, *26*(17), 2883.

Pine, S. R., Marshall, B., & Varticovski, L. (2008). Lung cancer stem cells. *Disease markers*, *24*(4-5), 257-266.

Ponti, D., Costa, A., Zaffaroni, N., Pratesi, G., Petrangolini, G., Coradini, D., ... & Daidone, M. G. (2005). Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer research*, *65*(13), 5506-5511.

Ridley, A. J. (2013). RhoA, RhoB and RhoC have different roles in cancer cell migration. *Journal of microscopy*, *251*(3), 242-249.

Sahai, E., & Marshall, C. J. (2002). RHO–GTPases and cancer. *Nature Reviews Cancer*, *2*(2), 133-142.

Sato, N., Fukui, T., Taniguchi, T., Yokoyama, T., Kondo, M., Nagasaka, T., ... & Minna, J. D. (2007). RhoB is frequently downregulated in non- small- cell lung cancer and resides in the 2p24 homozygous deletion region of a lung cancer cell line. *International journal of cancer*, *120*(3), 543-551.

Siegel, R. L., Miller, K. D., & Jemal, A. (2016). Cancer statistics, 2016. *CA: a cancer journal for clinicians*, *66*(1), 7-30.

Singh, S. K., Clarke, I. D., Terasaki, M., Bonn, V. E., Hawkins, C., Squire, J., & Dirks, P. B. (2003). Identification of a cancer stem cell in human brain tumors. *Cancer research*, *63*(18), 5821-5828.

Visvader, J. E., & Lindeman, G. J. (2012). Cancer stem cells: current status and evolving complexities. *Cell stem cell*, *10*(6), 717-728.

Walter, D., Satheesha, S., Albrecht, P., Bornhauser, B. C., D'Alessandro, V., Oesch, S. M., ... & Moch, H. (2011). CD133 positive embryonal rhabdomyosarcoma stem-like cell population is enriched in rhabdospheres. *PloS one*, *6*(5), e19506

Wang, S., Yan-Neale, Y., Fischer, D., Zeremski, M., Cai, R., Zhu, J., ... & Cohen, D. (2003). Histone deacetylase 1 represses the small GTPase RhoB expression in human nonsmall lung carcinoma cell line. *Oncogene*, *22*(40), 6204-6213.

Wang, P., Gao, Q., Suo, Z., Munthe, E., Solberg, S., Ma, L., ... & Gaudernack, G. (2013). Identification and characterization of cells with cancer stem cell properties in human primary lung cancer cell lines. *PLoS one*, *8*(3), e57020.

Weiswald, L. B., Bellet, D., & Dangles-Marie, V. (2015). Spherical cancer models in tumor biology. *Neoplasia*, *17*(1), 1-15.

Wicha, M. S., Liu, S., & Dontu, G. (2006). Cancer stem cells: an old idea—a paradigm shift. *Cancer research*, *66*(4), 1883-1890.

Winton, T., Livingston, R., Johnson, D., Rigas, J., Johnston, M., Butts, C., ... & Fry, W. (2005). Vinorelbine plus cisplatin vs. observation in resected non–small-cell lung cancer. *New England Journal of Medicine*, *352*(25), 2589-2597.

Zakaria, N., Satar, N. A., Halim, A., Hanis, N., Ngalim, S. H., Yusoff, N. M., ... & Yahaya, B. H. (2017). Targeting lung cancer stem cells: research and clinical impacts. *Frontiers in oncology*, *7*, 80.

Zhong, Y., Guan, K., Guo, S., Zhou, C., Wang, D., Ma, W., ... & Zhang, S. (2010). Spheres derived from the human SK-RC-42 renal cell carcinoma cell line are enriched in cancer stem cells. *Cancer letters*, *299*(2), 150-160.

Author Information

Fathiya RAHMI
Gaziantep University
Department of Biology, Gaziantep University
Şehitkamil/Gaziantep, Turkey
Contact e-mail: *fr11015@mail2.gantep.edu.tr*

Hiba KHAIR
Gaziantep University
Department of Biology, Gaziantep University
Şehitkamil/Gaziantep, Turkey

Işık Didem KARAGÖZ
Gaziantep University
Department of Biology, Gaziantep University
Şehitkamil/Gaziantep, Turkey