RESEARCH ARTICLE

Effects of a Phosphoinositide-3-Kinase Inhibitor on Anaplastic Thyroid Cancer Stem Cells

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Abstract

Background: Thyroidectomy, radioactive iodine therapy, chemotherapy, or their combination are treatments of choice for thyroid cancers. However, cancer stem cells (CSCs) may become resistant to therapy, and mutations in somatic genes affect radioiodine uptake. This study determined the effect of a phosphoinositide-3-kinase (PI3K) inhibitor on anaplastic thyroid CSCs. Materials and Methods: The magnetic-activated cell sorting assay was used for segregating CD133-positive CSCs from three anaplastic thyroid carcinoma (ATC) cell lines (C643, SW1736, and 8305C). After confirming the cells’ purity by flow cytometry, they were treated with 5, 10, 20, or 25 μM LY294002, a PI3K inhibitor, and then evaluated at 24 and 48 h. The sodium-iodide symporter (NIS) mRNA level was determined using the quantitative real-time polymerase chain reaction. NIS protein expression was evaluated using western blotting. Results: The PI3K inhibitor, at different concentrations and times, increased the NIS mRNA level (1.30-6.17-fold, P < 0.0001). If the NIS mRNA level in LY294002-treated CD133-positive CSCs was increased more than 2-fold, the NIS protein content was detectable. Conclusions: CD133-positive CSCs isolated from ATC cell lines expressed NIS mRNA and protein after PI3K inhibition. Our findings suggest that molecularly targeted CSC therapy may improve the treatment efficacy of aggressive cancers like ATC.

Keywords: Anaplastic thyroid carcinoma- CD133 antigen- Phosphoinositide-3-kinase- Sodium-iodide symporter

Introduction

At the present time, thyroidectomy followed by radioactive iodine (RAI) therapy, chemotherapy, or both are the treatments of choice for thyroid cancers (Fahrner et al., 2014; Fortuny et al., 2015). Most types of thyroid cancers, such as papillary and follicular thyroid cancers, can be treated successfully (Schmidbauer et al., 2017). However, advanced cancer types, such as anaplastic thyroid carcinoma (ATC), usually have no successful treatments. ATC is known as an aggressive, lethal, and recurrent malignancy that is resistant to routine treatments, especially RAI therapy (Pezzi et al., 2016).

Several factors are involved in effective RAI therapy including the sodium-iodide symporter (NIS) protein. This glycoprotein is formed in 13 putative transmembrane domains and is localized on the basolateral membrane of thyrocytes. It expedites iodine uptake in thyroid follicular cells and has a critical role in RAI therapy (Darrouzet et al., 2016; Ferrandino et al., 2016). The function of this protein can be affected by different factors, and by mutation in some somatic genes such as phosphoinositide-3-kinase (PI3K).

PI3K engages in several cellular activities including cell growth, proliferation, differentiation, motility, survival, and intracellular trafficking (Bozorg-Ghalati et al., 2015). Several studies reported that PI3K silencing can induce expression of NIS (Kogai et al., 2008). In addition to gene mutations (Bozorg-Ghalati et al., 2016), cancer stem cells (CSCs) in thyroid tumors are associated with tumor metastasis, recurrence, and drug resistance (Nagayama et al., 2016; Bozorg-Ghalati et al., 2017a,b). Due to the unknown effects of a mutant PI3K on NIS in thyroid CSCs, the present study examined CSCs expressing the CD133 surface marker in ATC cell lines, and surveyed NIS gene/protein expression after PI3K inhibition.

Materials and Methods

Culture of ATC cell lines

Three ATC cell lines (SW1736, C643, and 8305C) were used in this study. The SW1736 and C643 cell lines were graciously supplied by Dr. Vahid Haghpanah

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(Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran). The third cell line (8305C) was purchased from the National Cell Bank of Iran (Pasteur Institute of Iran, Tehran, Iran). All cells were cultured at 37°C under 5% CO₂, in RPMI 1640 GlutaMAX™ medium (BioWest, Nuaille, France) and supplemented with 10% fetal bovine serum (Gibco™, EU-Approved, South American), 1% penicillin-streptomycin, and 1% non-essential amino acids (BioWest).

**Quantitative real-time polymerase chain reaction (qRT-PCR)**

Samples were loaded in triplicate into 48-well optical plates of the StepOne PCR thermal cycler system, version 2.3 (Applied Biosystems, Lincoln, NE, USA). Each PCR reaction mixture contained 100 ng of cDNA, RealQ PCR 2x Master Mix SYBR Green high ROX® (Ambicon, Stenhuggervej, Denmark), PI3K or NIS primers (Macrogen, Seoul, South Korea), and RNAase/DNAase-free water (Thermo Scientific). For each sample, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene for normalizing the expression of PI3K and NIS. The primer sequences are given in Table 1.

**Western blotting**

Treated cells were lysed in lysis buffer consisting of 50 mM Tris (pH 8.0) (Merck, Darmstadt, Germany), 150 mM NaCl (Merck), 1% Triton X-100 (Sigma-Aldrich), and 10 μg/mL aprotinin (Sigma-Aldrich). Protein concentrations were measured by the Bradford assay (Bio-Rad, Hercules, CA, USA). Total proteins (15 μg), the PageRuler ladder (Thermo Scientific), and healthy human thyrocyte lysate (as a positive control) were loaded onto a 10% sodium dodecyl sulfate–polyacrylamide gel and electrophoresed (Mini-PROTEAN Tetra Vertical Electrophoresis Cell, Bio-Rad, Marnes-la-Coquette, France). The bands were transferred (4°C, overnight) onto a Protran Nitrocellulose Transfer Membrane (Sigma-Aldrich). The membrane was blocked (90 min at room temperature) in 5% skimmed milk (Merek) in washing buffer (10 mM Tris–HCl [pH 8.0], 150 mM NaCl, and 0.05% Tween® 20 [Merck]). After washing, it was incubated (1 h at room temperature) with an NIS antibody diluted 1:200 (ab17775, Abcam, Cambridge, UK) in 1% bovine serum albumin (Sigma-Aldrich)-washing buffer. Then, the washing steps were repeated and the membrane was incubated (45 min at room temperature) with horseradish peroxidase-conjugated goat anti-mouse IgG (ab97023, Abcam), diluted 1:2000 in 1% bovine serum albumin-washing buffer. Finally, the bands were visualized with 3,3’-diaminobenzidine (Sigma-Aldrich).

**Statistical analysis**

Graphpad Prism version 6.01 analytic software (Graphpad, San Diego, CA, USA) and the one-way analysis of variance test were used to determine differences in the expression levels of PI3K and NIS mRNA. A P value electrophoresis. cDNA was synthesized by using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA).

**Flow cytometry**

A suspension (100 μL) containing 10⁶ cells/mL was prepared. Then, 10 μL of the CD133 antibody (Miltenyi Biotec) were added, mixed well, and incubated at 4°C for 10 min. Subsequently, the cells were washed with MACS buffer and centrifuged at 300 × g for 10 min. Finally, the supernatant was aspirated and a suitable amount of MACS buffer added for analysis of the cells by flow cytometry. Then, 10 μL of the CD133 antibody (Miltenyi Biotec) were added, mixed well, and incubated at 4°C for 15 min under a low rotator speed. Then, the washes were washed with MACS buffer, centrifuged at 300 × g for 10 min, and resuspended in MACS buffer (500 μL). LS columns (Miltenyi Biotec) that were fixed on the MACS separator magnet, were rinsed with MACS buffer (3 mL) and the cell suspensions were infused. After gathering the effluent from each LS column, they were removed from the MACS separator and placed into a new collection tube. Finally, by applying the MACS buffer (5 mL) and piston, the magnetically-marked CD133-positive CSCs were obtained.

**Treatments**

CD133-positive CSCs isolated from the three ATC cell lines (C643, SW1736, and 8305C) were treated with 5, 10, 20, or 25 μM LY294002 (a PI3K inhibitor) (Chemietek, Indianapolis, IN, USA) and 5 μg/mL bovine thyroid-stimulating hormone (Sigma-Aldrich) for 24 and 48 h. The treatment of 24 samples was repeated two times. Cells cultured without the inhibitor were used for the control group.

**RNA isolation and cDNA synthesis**

Total RNA was extracted from the treated cells according to the YTA Total RNA Extraction Mini Kit protocol (Yekta Tajhiz Azma, Tehran, Iran). The purity, quantity, and integrity of total RNA were determined by ultraviolet spectrophotometry and agarose gel electrophoresis. A P value according to the YTA Total RNA Extraction Mini Kit protocol (Yekta Tajhiz Azma, Tehran, Iran). The purity, quantity, and integrity of total RNA were determined by ultraviolet spectrophotometry and agarose gel electrophoresis. cDNA was synthesized by using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA).

**Magnetic-activated cell sorting (MACS)**

CD133-positive CSCs were isolated from the three ATC cell lines by using the MACS method. A MACS® human CD133 Microbead Kit-Tumor Tissue (Miltenyi Biotec, Bergisch Gladbach, Germany) was used according to the manufacturer's protocol. After cultivation of the cell lines, they were harvested by trypsin-EDTA (Sigma-Aldrich) and centrifuged at 300 × g for 10 min. The pellets were resuspended in 60 μL of MACS buffer (Miltenyi Biotec), 20 μL of FcR blocking reagent (Miltenyi Biotec), and 20 μL of CD133 microbeads, and incubated at 4°C for 15 min under a low rotator speed. Then, the washes were washed with MACS buffer, centrifuged at 300 × g for 10 min, and resuspended in MACS buffer (500 μL). LS columns (Miltenyi Biotec) that were fixed on the MACS separator magnet, were rinsed with MACS buffer (3 mL) and the cell suspensions were infused. After gathering the effluent from each LS column, they were removed from the MACS separator and placed into a new collection tube. Finally, by applying the MACS buffer (5 mL) and piston, the magnetically-marked CD133-positive CSCs were obtained.
Results

Verification of CSC purity

CD133-positive CSCs with 92.2, 71.6, and 96.9% purity were isolated from the C643, SW1736, and 8305C cell lines, respectively (Figure 1).

Evaluation of PI3K and NIS mRNA expression in CD133-positive CSCs segregated from ATC cell lines after treatment with LY294002

Analysis of the qRT-PCR data showed that, compared with the level before treatment (control group), the expression of NIS mRNA increased in the CD133-positive CSCs separated from C643 (Figure 2A), SW1736 (Figure 2C), and 8305C (Figure 2E) cells after 24 and 48 h of treatment with 5, 10, 20, or 25 μM LY294002. The results also indicated that the expression of PI3K mRNA decreased in the CD133-positive CSCs separated from C643 (Figure 2B), SW1736 (Figure 2D), and 8305C (Figure 2F) cells after incubation with these four concentrations of LY294002. The data are summarized in Table 2.

Increased levels of NIS protein expression in CD133-positive CSCs after treatment with LY294002

To assess the effects of LY294002 on NIS protein levels in CD133-positive CSCs, western blotting was performed. The results showed that CD133-positive CSCs isolated from C643, SW1736, and 8305C cells treated with LY294002 were capable of expressing the NIS protein compared with pre-treatment. In addition, various LY294002 concentrations caused different changes in expression of the NIS protein (Figure 3).

Discussion

In this investigation, we studied anaplastic thyroid CSCs with the CD133 surface marker. CSCs have self-renewal ability and generate diverse cancer cells that are linked to tumor recurrence and drug resistance (Franco et al., 2016). They are identified by the expression of multiple genes (Shimamura et al., 2014) and various surface markers such as CD133 (Grasso et al., 2016; Zhang et al., 2016). These cells are usually present in low numbers (0.5-18%) in tumor tissues or cell lines (Schmohl and Vallera, 2016). Because of their low numbers in ATC cell lines, and for raising the purity of isolated cells, we used the MACS method and repeated the sorting assay for the first flow-through with new MACS columns until reaching a high purity (more than 70%) of CD133-positive CSCs.

Table 1. Accession Numbers, Primer Sequences, and Expected Product Lengths of PI3K, NIS, and GAPDH

| Gene  | Accession No. | Primer sequence (5’ to 3’) | Product length(bp) |
|-------|---------------|----------------------------|--------------------|
| PI3K  | NM_006218.2   | (F) AACCTCAGGCTTGAGAG (R) GAAGTTGATGATACCTGTC | 157                |
| NIS   | NM_000453.2   | (F) CTATGGCCTCAAGTTCCTCT (R) CGTGGCTACAATGTACTGC | 178                |
| GAPDH | NM_002046.5   | (F) GCTCTCTGTGCTCCTGTTTC (R) CGACCAATCGTGGACTCC | 114                |

F, forward; R, reverse
Table 2. Fold Changes in PI3K and NIS mRNA Levels Using the Livak Method in CD133-Positive Cells Isolated from the ATC Cell Lines after 24 and 48 h Treatment with LY294002.

| Cell type | Concentration of LY294002 μM | Time | PI3K gene expression | NIS gene expression |
|-----------|-------------------------------|------|----------------------|---------------------|
| CD133pos cells isolated from C643 cell line | 5 | 24 hours | 0.80±0.01 | 3.56±0.11 |
| | 10 | 24 hours | 0.74±0.02 | 3.61±0.11 |
| | 20 | 24 hours | 0.23±0.0 | 5.02±0.36 |
| | 25 | 24 hours | 0.84±0.04 | 2.41±0.18 |
| | 5 | 48 hours | 0.74±0.0 | 4.44±0.23 |
| | 10 | 48 hours | 0.66±0.02 | 4.48±0.13 |
| | 20 | 48 hours | 0.30±0.0 | 6.17±0.11 |
| | 25 | 48 hours | 0.85±0.03 | 2.85±0.09 |
| CD133pos cells isolated from SW1736 cell line | 5 | 24 hours | 0.62±0.01 | 3.80±0.0 |
| | 10 | 24 hours | 0.58±0.01 | 5.57±0.32 |
| | 20 | 24 hours | 0.24±0.0 | 5.51±0.2 |
| | 25 | 24 hours | 0.77±0.03 | 2.09±0.10 |
| | 5 | 48 hours | 0.41±0.0 | 3.48±0.05 |
| | 10 | 48 hours | 0.38±0.0 | 5.42±0.45 |
| | 20 | 48 hours | 0.11±0.0 | 5.66±0.21 |
| | 25 | 48 hours | 0.82±0.02 | 2.19±0.14 |
| CD133pos cells isolated from 8305C cell line | 5 | 24 hours | 0.65±0.11 | 2.91±0.21 |
| | 10 | 24 hours | 0.39±0.07 | 3.38±0.25 |
| | 20 | 24 hours | 0.07±0.01 | 5.85±0.21 |
| | 25 | 24 hours | 0.85±0.04 | 1.68±0.09 |
| | 5 | 48 hours | 0.41±0.07 | 2.72±0.05 |
| | 10 | 48 hours | 0.24±0.03 | 3.12±0.14 |
| | 20 | 48 hours | 0.09±0.02 | 4.52±0.21 |
| | 25 | 48 hours | 0.76±0.11 | 1.34±0.06 |

Analysis of the qRT-PCR data revealed that various concentrations (5, 10, 20, and 25 μM) and times (24 and 48 h) of LY294002 treatment had different effects on PI3K and NIS mRNA levels. We found that 20 μM of this inhibitor was most effective at raising diminishing NIS and PI3K mRNA levels, respectively. LY294002 at 25 μM had the least influence on the expression of NIS and PI3K. This indicates that an elevated concentration of the PI3K inhibitor limited its action on NIS expression. We believe that other factors may be involved in this mechanism and should be studied in the future. In addition, although all cells isolated from the three distinct ATC cell lines (C643, SW1736, and 8305C) were treated under identical conditions, the fold inductions of NIS were not the same. These data illustrate that intensifying activity of the PI3K/Akt pathway in CSCs plays a dominant role in activating NIS. Our findings are consistent with previous studies (Lu et al., 2012; Serrano-Nascimento et al., 2014).

Recent studies revealed that PI3K activation can modify NIS on the cell surface and reduce all-trans retinoic acid and hydrocortisone-influenced glycosylated NIS protein expression, and NIS-mediated RAI uptake (Knoostman et al., 2004; Li et al., 2006; Ohashi et al., 2009; Wu et al., 2005). Based on our PI3K expression findings, NIS protein expression was assessed in the treated CSCs.

Overall, our data indicate that expression of the NIS protein in CSCs is very low and thus not a major factor in the iodide uptake process. However, we believe that focusing on CSCs in a targeted therapy manner is an effective way to create successful treatments for ATC patients. Our study shows that these cells express NIS mRNA and protein as a thyroid differentiation marker. It may be possible to eradicate these cells with several strategies such as the use of toxins (Waldron et al., 2014; Waldron and Vallera, 2013), immunotoxins (Ohlfest et al., 2013), monospecific anti-CSCs antibodies (Damek-Poprawa et al., 2011), anti-CSC conjugated nanoparticles (Swaminathan et al., 2013), anti-CSCs bi-specific antibodies (Huang et al., 2013), trispecific natural killer engagers (Schmohl et al., 2016; Waldmann, 2014), and aptamers (Shigdar et al., 2013).

In conclusions, our results reveal that CD133-positive CSCs isolated from the 8305C cell line and treated with 25 μM LY294002 for 24 and 48 h did not express increased NIS protein even though the mRNA levels were increased 1.68 ± 0.09- and 1.34 ± 0.06-fold at 24 and 48 h, respectively. The mRNA levels of other CD133-positive CSCs that expressed levels of this protein, were increased more than 2-fold. This indicates that expression of the NIS protein is linked to its mRNA levels. Other factors may also be involved in the process of protein expression that need to be clarified in future studies.
could induce expression of NIS mRNA and protein. This may improve the efficacy of RAI therapy in aggressive cancers like ATC.

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**Conflict of interest**

The authors declare no conflict of interest.

**References**

Bozorg-Ghalati F, Hedayati M (2015). Relationship between PI3K mutation and sodium-iodide symporter in anaplastic thyroid carcinoma. *Am J Cancer Sci*, 4, 63-77.

Bozorg-Ghalati F, Hedayati M (2016). BRAF mutation and its effects on radiiodine uptake in patients with anaplastic thyroid carcinoma. *Am J Cancer Sci*, 5, 22-33.

Bozorg-Ghalati F, Hedayati M, Dianatpour M (2017a). CD133 as a biomarker of thyroid cancer stem cells. *Int J Curr Res*, 9, 51284-91.

Bozorg-Ghalati F, Hedayati M, Dianatpour M (2017b). Are surface markers of cancer stem cells reliable factors for cancer treatment follow-up?. *Comp Clin Pathol*, 26, 981-3.

Damek-Poprawa M, Volgina A, Korostoff J, et al (2011). Targeted inhibition of CD133+ cells in oral cancer cell lines. *J Dent Res*, 90, 638-45.

Darrouzet E, Graslin F, Marcellin D, et al (2016). A systematic evaluation of sorting motifs in the sodium-iodide symporter (NIS). *Biochem J*, 473, 919-28.

Fahrner R, Übersax L, Mettler A, Berger S, Seiler CA (2014). Pediatric thyroid surgery is safe experiences at a tertiary surgical center. *Swiss Med Wkly*, 144, w13939.

Ferrandino G, Nicola JP, Sánchez YE, et al (2016). Na+ coordination at the Na2 site of the Na+/I-symporter. *Proc Natl Acad Sci USA*, 113, E5379-88.

Fortuny JV, Guigard S, Karenovics W, Triponez F (2015). Surgery of the thyroid: recent developments and perspective. *Swiss Med Wkly*, 145, w14144.

Franco S, Szczesna K, Iliou MS, et al (2016). In vitro models of cancer stem cells and clinical applications. *BMC Cancer*, 16, 738.

Grasso C, Anaka M, Hofmann O, et al (2016). Iterative sorting reveals CD133+ and CD133- melanoma cells as phenotypically distinct populations. *BMC Cancer*, 16, 726.

Huang J, Li C, Wang Y, et al (2013). Cytokine-induced killer (CIK) cells bound with anti-CD3/anti-CD133 bi-specific antibodies target CD133 (high) cancer stem cells in vitro and in vivo. *Clin Immunol*, 149, 156-68.

Knoostman KAB, Cho JY, Ryu KY, et al (2004). Signaling through 3',5'-cyclic adenosine monophosphate and phosphoinositide-3 kinase induces sodium/iodide symporter expression in breast cancer. *J Clin Endocrinol Metab*, 89, 5196-203.

Kogai T, Sajid-Crockett S, Newmarch LS, Liu YY, Brent GA (2008). Phosphoinositide-3-kinase inhibition induces sodium/iodide symporter expression in rat thyroid cells and human papillary thyroid cancer cells. *J Endocrinol*, 199, 243-52.

Li SY, Rong M, Grieu F, Iacopetta B (2006). PI3KCA mutations in breast cancer are associated with poor outcome. *Breast Cancer Res Treat*, 96, 91-5.

Liu YY, Zhang X, Ringel MD, Jhiang SM (2012). Modulation of sodium iodide symporter expression and function by LY294002, Akti-1/2 and Rapamycin in thyroid cells. *Endocr Relat Cancer*, 19, 291-304.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, 25, 402-8.

Nagayama Y, Shimamura M, Mitsutake N (2016). Cancer stem cells in the Thyroid. *Front Endocrinol*, 7, 20.

Ohashi E, Kogi T, Kagechika H, Brent GA (2009). Activation of the PI3 kinase pathway by retinoic acid mediates sodium/iodide symporter induction and iodide transport in MCF-7 breast cancer cells. *Cancer Res*, 69, 3443-50.

Ohlfest JR, Zellmer DM, Panyam J, et al (2013). Immunotoxin targeting CD133+ breast cancer cells. *Drug Deliv Transl Res*, 3, 195-204.

Pezzi TA, Mohamed AS, Sheu T, et al (2016). Relationship between radiation therapy dose and overall survival in anaplastic thyroid cancer: analysis of the national cancer data base. *Int J Radiat Oncol Biol Phys*, 96, E361.

Schimidbauer B, Menhart K, Hellwig D, Grosse J (2017). Differentiated thyroid cancer treatment: state of the art. *Int J Mol Sci*, 18, E1292.

Schmolch JU, Felices M, Taras E, Miller JS, Vallera DA (2016). Enhanced ADCC and NK cell activation of an anti-carcinoma bi-specific antibody by genetic insertion of a modified IL-15 cross-linker. *Mol Ther*, 24, 1312-22.

Schmolch JU, Vallera DA (2016). CD133, selectively targeting the root of cancer. *Toxins*, 8, 165-84.

Serrano-Nascimento C, da Silva Teixeira S, Nicola JP, et al (2014). The acute inhibitory effect of iodide excess on sodium/iodide symporter expression and activity involves the PI3K/Akt signaling pathway. *Endocrinology*, 155, 1145-56.

Shigdar S, Qiao L, Zhou SF, et al (2013). RNA aptamers targeting cancer stem cell marker CD133. *Cancer Lett*, 330, 84-95.

Shimamura M, Nagayama Y, Matsuse M, Yamashita S, Mitsutake N (2014). Analysis of multiple markers for cancer stem-like cells in human thyroid carcinoma cell lines. *Endocr J*, 61, 481-90.

Swaminathan SK, Roger E, Toti U, et al (2013). CD133-targeted paclitaxel delivery inhibits local tumor recurrence in a mouse model of breast cancer. *J Control Release*, 171, 280-7.

Waldmann TA (2014). Interleukin-15 in the treatment of cancer. *Expert Rev Clin Immunol*, 10, 1689-701.

Waldron NN, Vallera DA (2013). An old idea tackling a new problem: Targeted toxins specific for cancer stem cells. *Antibodies*, 2, 82-92.

Waldron NN, Barsky SH, Dougherty PR, Vallera DA (2014). Bi-specific EpCAM/CD133-targeted toxin is effective against carcinoma. *Target Oncol*, 9, 239-49.

Wu G, Xing M, Mambo E, et al (2005). Somatic mutation and human papillary thyroid cancer cells. *Cancer Res Treat*, 9, R609-16.

Zhang SS, Huang ZW, Li LX, Fu JJ, Xiao B (2016). Identification of CD200+ colorectal cancer stem cells and their gene expression profile. *Oncol Rep*, 36, 2252-60.