SUPPLEMENT

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Investigation of the expression of RIF1 gene on head and neck, pancreatic and brain cancer and cancer stem cells

Abstract

Purpose: Recent studies have shown that cancer stem cells are resistant to chemotherapy. The aim of this study was to compare RIF1 gene expression in head and neck, pancreatic cancer and glioma cell lines and the cancer stem cells isolated from these cell lines.

Methods: UT-SCC-74 from Turku University and UT-SCC-74B primary tumor metastasis and neck cancer cell lines, YKG-1 glioma cancer cell line from RIKEN, pancreatic cancer cell lines and ASPC-1 cells from ATCC were grown in cell culture. To isolate cancer stem cells, ALDH-1 for UT-SCC-74 and UT-SCC-74B cell line, CD-133 for YKG-1 cell line and CD-24 for ASPC-1 cell line, were used as markers of cancer stem cells. RNA isolation was performed for both cancer lines and cancer stem cells. RNAs were converted to cDNA. RIF1 gene expression was performed by qRT-PCR analysis. RIF1 gene expression was compared with cancer cell lines and cancer stem cells isolated from these cell lines. The possible effect of RIF1 gene was evaluated.

Results: In the pancreatic cells, RIF1 gene expression in the stem cell-positive cell line was 256 time that seen in the stem cell-negative cell line.

Conclusion: Considering the importance of RIF1 in NHEJ and of NHEJ in pancreatic cancer, RIF1 may be one of the genes that plays an important role in the diagnoses and therapeutic treatment of pancreatic cancer. The results of head and neck and brain cancers are inconclusive and further studies are required to elucidate the connection between RIF1 gene and these other types of cancers.

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Cancer is a disease that has been responsible for many deaths over the past century. One of the most deadly cancers is a pancreatic cancer, which is known for its poor prognosis and lethality. This study focused on pancreatic cancer in ASPC-1 cell line and two other cancers, brain (YKG-1) and head and neck (UT-SCC 74A), to attempt to find a commonality for these three specific type of cancers to aid in diagnosis and treatment.

Over the past several years, cancer research has expanded to a new area; cancer stem cells. Research in this area has shown that even if a tumour is surgically removed from a patient or chemotherapy is applied to the patient, it is highly possible that the cancer cells will continue to reproduce inside the patient. This research is targeted at stem cells in an attempt to eliminate the possibility of cancer cells reproducing in patients with a history of cancer by proposing efficient and permanent ways of the disease once for all. Cancer stem cells (CSC) are a sub-population of cancer cells that are thought to be the primary tumorigenic cells [5]. CSCs have been identified in many solid tumors, including brain, breast, lung, prostate, ovary, and colorectal cancers, and they are thought to be responsible for these aggressive and recurrent phenotypes [6]. Since there are some issues about specific genes of stem cells, these cells can be transformed for study. One of the genes that causes transformation is RIF1 (RAP1 Interacting Factor 1), which is responsible for protein coding sequence, avoids chemotherapy drugs and also causing transformation and histone modification and methylation.

Previous studies have shown that RIF1 has a significant place in ESC stability and DNA DSB repair mechanisms [1,2]. High expression levels of RIF1 are associated with transformation in ESC's [3]. RIF1 also promotes NHEJ in stem cells; thus, it avoids chemotherapy in cancer stem cells [4]. Many studies have done with RIF1 in breast and other cancers; however, pancreatic head and neck and brain cancer have been less studied. The purpose of this research was to investigate the link between RIF1 gene expression and cancer stem cells for ASPC-1 cell line in pancreatic cancer, as well as for YKG-1 in brain cancer and UT-SCC 74A in head and neck cancers.

Materials and Methods

Cell lines and Cell culture

Head and neck squamous cell carcinoma cell line (UT-SCC 74A; University of Turku Squamous Cell Carcinom) was kindly provided by Prof. Reidar Grenman (Department of Otorhinolaryngology, University of Turku and Turku

University Hospital, Turku, Finland). Glioblastoma cell line (YKG1) was obtained from RIKEN. Pancreatic cancer cell line (ASPC-1) was obtained from the American Type Culture Collection, Manassas, VA, USA.

UT-SCC-74A and YKG-1 were cultured in Dulbecco's Modified Eagle's Medium (Hyclone, Logan, UT, USA) supplemented with 10% heat inactivated fetal bovine serum (Hyclone), 1% L-glutamine 200 mM (Hyclone), 1% penicillin/streptomycin 100 U/mL (Hyclone), and 2.5 µg/mL plasmocin prophylactic (Invivogen, Toulouse, France). ASPC-1 was cultured in RPMI1640 (SH30027.01) supplemented with 10% heat inactivated fetal bovine serum (Hyclone), 1% L-glutamine 200 mM (Hyclone), 1% penicillin/streptomycin 100U mL (Hyclone) and 2.5 µg/mL plasmocin prophylactic (Invivogen, Toulouse, France). Cultures were maintained in a 5% CO₂ humidified incubator at 37°C [7].

Isolation and Characterization of cancer stem cells (CSCs) from cancer cells

Cancer stem-cells were isolated from the head and neck cancer cell line (UT-SCC 74A) using ALDH1 as a biomarker, from the glioblastoma cell line (YKG-1) using CD-133 as a biomarker and from the pancreatic cancer cell line (ASPC-1) using CD-24 as a biomarker. Magnetic activated cell sorting (MACS) was used and sphere formation was confirmed.

UT-SCC-74A, YKG-1 and ASPC-1 cells were harvested by trypsinization. The cell suspension in PBS was incubated with mouse monoclonal antibody ALDH1 (Abcam, Cambridge, USA; ab105920) for UT-SCC-74A, CD-133 for YKG-1 and anti-human CD24-BV421 for ASPC-1. After incubation with magnetic beads coated with goat antimouse IgG (NEB), the bead-cell complex were isolated in a magnetic rack and then cultured in stem cell medium. Stem cell medium content was DMEM High Glucose medium supplemented with 1% N2 Supplement (Gibco), 2% B27 Supplement (Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin (Hyclone), 100 ng/mL epidermal growth factor (Abcam, Cambridge, MA, USA),) and 50 ng/mL fibroblast growth factor (Abcam, Cambridge, MA, USA).

Using magnetic beads, ALDH1 (+) and ALDH1 (-) cells were isolated from the primary tumour cell line (UTSCC-74A). CD-133 (+) and CD-133 (-) cells were isolated from the YKG-1 cell line. CD-24(+) and CD-24(-) cells were isolated from the ASPC-1 cell line.

RNA isolation and qRT-PCR

ALDH1 (+) and ALDH1 (-), CD-133 (+) and CD-133 (-) and CD-24(+) and CD-24(-) cells were then seeded in a 6 well plate for RNA isolation. RNA was collected from the isolated stem cells. Total RNA was isolated from UT-SCC 74A, YKG-1 and ASPC-1 cancer stem cell lines using Trizol (Invitrogen) according to manufacturer's instructions. Total RNA (1µg) was used for cDNA synthesis with Thermo Scientific RevertAid First Strand cDNA Synthesis Kit. The two selected primers used are B-actin and RIF-1 (Table 1).

The Beta-actin gene was used as endogenous control for normalization. The annealing temperatures and numbers of amplification cycles of these two genes in the PCR assay are shown in Table 1. Real-time PCR was run with a program consisting of a 95°C denaturation for 5 min, a 95°C denaturation for 30 s, followed by a 59°C annealing for 30 s and a 72°C extension for 60 s by SYBR-green (Applied Biosystems® StepOnePlus™ Real-Time PCR Systems).

Results

To obtain optimum amounts of the cancer stem cells, we waited for seven days to continue the process (Fig 1). After successfully obtaining the stem cells, qRT-PCR was done with both cancer stem cell-positive and cancer stem cell-negative cell lines. Gene expression was significantly increased in the pancreatic cancer stem cell-positive cell line in comparison with the cancer stem cell-negative cell line. For the stem cell-positive lines, RIF1 gene expression increased 256-fold over the stem cell-negative cell line. RIF's one of the most distinct effects is found on pancreatic cancer stem cell line (Fig 2). NHEJ repair mechanism is the most important effect of RIF in pancreatic cancer.

Results for brain cancer (YKG1) (Fig 3) and head and neck cancer stem cell lines (74A) were unclear (Fig 4).

Discussion

The role of RIF1 gene on brain cancer and head and neck cancer remains unclear; however, the significant increase in RIF1 expression (a 256 times increase obtained with pancreatic cancer stem cell-positive cell line compared to stem

ASPC-1 Pancreatic cancer cell (10x)

YKG-1 brain cancer cell (10x)

UT-SCC-74A head and neck cancer (10x)

CD24 (+) cells formed sphere 7.day (40x)

ALDH1(+) cells formed sphere 7.day (40x)

CD133 (+) cells formed sphere 7.day (40x)

Figure 1. Sphere formation assay. ALDH1(+), CD24 (+) and CD133 (+) cells formed sphere.

Figure 2. RIF1 mRNA gene expression in pancreatic cancer stem cell (ASPC-1)-positive and -negative lines.

cell-negative cell line) might signal a new approach in the study of pancreatic cancer using the ASPC-1 cell line. This significant increase may lead to new opportunities for treatment and diagnosis of pancreatic cancer. Additional research is required.

This study also aimed to determine whether RIF1 could be useful in the treatment and diagnosis other type of cancers. RIF1 gene is responsible for the DSB repair mechanism, avoiding chemotherapy, transformation in stem cells and epigenetic interactions; thus, this seems a promising area of research that has not been well studied.

Even with a significant of increase in expression of the RIF1 gene in the pancreatic cancer cell line, other types of cancers, such as brain and head and neck, did not show a comparable increase. It is too early to say definitively whether or not RIF1 gene expression is a common part of all type of cancers.

The results of this study are supported by several previously published studies. One study claimed that NHEJ repair mechanism is observed very frequently in pancreatic cancer and another study showed that RIF1 gene increases the efficiency of NHEJ repair mechanism [3,4]. The next step to elucidate the role of RIF1 gene could be to use siRNA's to silence the gene as and then observe the effect in pancreatic cancer cells.

Figure 3. RIF1 mRNA gene expression in brain cancer stem cell (YKG1)-positive and -negative lines.

Figure 4. RIF1 mRNA gene expression in head and neck cancer stem cell (74A)-positive and -negative lines.

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