SUPPLEMENT

Ebru Evran^{1*}
Hilal Şahin^{1*}
Kübra Akbaş²
Sadik Çiğdem²
Esra Gündüz²

¹Turgut Ozal University Faculty of Medicine, Ankara, Turkey

²Department of Medical Genetics, Turgut Ozal University Faculty of Medicine, Ankara, Turkey

*Equal contribution first authorship

Investigation of MACC1 Gene Expression in Head and Neck Cancer and Cancer Stem Cells

Abstract

Purpose: By investigating the MACC1 gene (metastasis-associated in colon cancer 1) in cancer stem cells (CSC) resistant to chemotherapy and in cancer stem cells (CSC) resistant to chemotherapy and in cancer cells (CS) sensitive to chemotherapy we determined a steady expression in both types of cells in head and neck cancer. In conformity with the result we examined if this gene could be a competitor gene for chemotherapy. According to literature, the MACC1 gene shows a clear expression in head and neck cancer cells [1]. Here we examined MACC1 expression in CSC and investigated it as a possible biomarker.

Methods: Our experiments were performed in the UT -SCC -74 in primary head and neck cancer cell line. We examined the MACC -1 gene expression by Real Time PCR from both isolated CSC and CS.

Results: Expression of MACC -1 gene of cancer stem cells showed an two-fold increase compared with cancer cells. Based on the positive expression of MACC1 in both CS and CSC, this gene may serve as a potential biomarker in head and neck cancer. By comparing the results of this study with the novel features of MACC1, two important hypotheses could be examined. The first hypothesis is that MACC1 is a possible transcription factor in colon cancer, which influences a high expression of CSC in head and neck and affects the expression of three biomarkers of the CSC control group biomarkers. The second hypothesis is that the positive expression of MACC1 in patients with a malignant prognosis of tongue cancer, which belongs to head and neck cancer types, operates a faster development of CSC to cancer cells.

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Correspondence to:

Esra Gunduz, DMD, PhD

Turgut Ozal University Faculty of Medicine, Department of Medical Genetics

Yunus Emre Mah. Takdir Cad. No:5, Yenimahalle, Ankara, 06170, TURKEY

E-mail: esragunduz@outlook.com

Cancer cells are growing and dividing cells that are rapid and irregular. Although cancer cells are frequently generated in the body, cancer disease occurs when the immune system fails to recognize and/or destroy them [2]. The reason of this inability is due to a weakness in the immune system and recent research has shown that the failure to recognize cancer cells is caused by the lack of particular co-stimulated molecules that help antigens to react with lymphocytes [3].

Cancer stem cells have the capacity to give rise to all kind of cells of a particular cancer sample. These cells are in connection with the other normal stem cells. Cancer stem cells (CSC) may generate tumours through the stem cell processes of self-renewal and differentiation into multiple cell types. Such cells are hypothesized to persist in tumours as a distinct population and cause relapse and metastasis by giving rise to new tumours. Development of specific therapies projected at cancer stem cells holds hope for improvement in survival rates and quality of life for cancer patients, particularly for patients with metastatic cancer disease.

The effect of cancer treatment in the first stages of testing is often measured by the ablation fraction of tumour mass (fractional kill). As CSCs form a small proportion of the tumour, this may not necessarily choose for drugs that act specifically on the stem cells. The theory proposes that conventional chemotherapies kill differentiated or differentiating cells, which form the development of the tumour but do not generate new cells. A population of CSCs, which gave rise to it, could remain untouched and cause relapse [4].

In different tumour subtypes, tumours are composed of cells with the differentiation and proliferation capacity, because these cells within the tumour population exhibit functional heterogeneity [5].

MACC1 (metastasis-associated in colon cancer 1) is a gene associated with colon cancer that functions as a key modulator of the hepatocyte growth factor (HGF; MIM 142409)-HGF receptor (HGFR, or MET; MIM 164860) pathway. This pathway plays an active role in cellular growth, epithelial-mesenchymal transition, angiogenesis, cell motility, invasiveness and metastasis. The expression of MACC1 in colon cancer (MIM 114500) counts as a novel biomarker for metastasis formation and also for survival free of metastasis [6]. MACC1 expression is also found in gastric cancer, tongue squamous cell carcinoma, renal cell carcinoma, breast cancer, lung cancer, ovarian cancer, cervical cancer and hepatocellular carcinoma.

The MACC1 gene was firstly identified by a genome-wide search for several expressed genes in human colon cancer

tissues, metastases and normal tissues. The expression was examined in primary colon cancers without any metastases and the negative and positive prediction for not synchronous metastasis was correct in 80% and 74% of cases, respectively. Patients with low MACC1 expression had a 5-year-survival of 80% in contrast to patients with high expression with a survival of only 15% [7]. This gene activates the HGF/Met transcription pathways by inducing migration, invasion and proliferation (in cell cultures). The presence of hepatocyte growth factor (HGF) results in translocation of MACC1 into the nucleus, where it links with the promoter of the receptor tyrosine kinase, Met [8-11].

Materials and Methods

Isolation of Cancer Stem Cells

Experiments were performed in the UT -SCC -74 in primary head and neck cancer cell line. Head and neck squamous cell carcinoma cell line (UT-SCC 74A; University of Turku Squamous Cell Carcinoma) was kindly provided by Prof. Reidar Grenman (Department of Otorhinolaryngology, University of Turku and Turku University Hospital, Turku, Finland). To isolate the cancer cells and CSC, a 12 well plate

Figure 1. Gradient PCR results for the MACC1 gene.

with 8,000,000 cells was used. ALDH - 1 surface markers were used to separate the CSC from this cell population [12].

RNA isolation and cDNA synthesis

After 48 hours, RNA isolation of these two cells types was performed. cDNA was prepared from this RNA sequence as described previously [12].

Quantitative real-time RT-PCR

Finally, we examined the MACC -1 gene expression using Real Time PCR [12]. The primers designed for MACC1 gene are shown in Table 1.

TABLE 1. Primers for MACC1 gene mRNA expression

Forward	GTTTCATCACCGGAGGAATCAAAAG
Reverse	GAATTGCACAAAGTATGTCTGAAGC

Results

Primers were optimized for MACC1 gene and PCR products were detected at the same size as expected at 66°C (Figure 1). Real Time PCR analysis was performed to detect the MACC 1 gene of cancer stem cells and cancer cells. Our results displayed a two-fold increase in MACC1 gene expression of CSC compared with cancer cells (Figure 2).

Discussion

According to literature, MACC1 overexpression has been found in head and neck cancers, specially in tongue squamous cell carcinoma [13]. Results of our experiments showed an induced MACC1 expression of head and neck CSC; even in comparison with non-CSC cancer cells it showed a two-fold increase. It has been reported in the literature that MACC1 induces pathways in CSC by expression of the pluripotency markers, Nanog and Oct4. Our results of a two-fold increase in MACC1 in CSC support these data and support our prediction that MACC1 may be a novel therapeutic target.

Different single nucleotide polymorphisms (SNPs) have been detected with 16 SNPs in the coding sequence, one leads to an exchange of arginine to threonine (R804T), an other one

Figure 2. Quantitative Real-Time PCR results for the MACC1 gene.

drive to a premature stop codon [14]. Many tyrosine residues with high ability of phosphorylation were found in MACC1; these are known as binding structures for SH2 domain comprising proteins, such as tyrosine kinases, phosphatases and adapter proteins [15] [16].

MACC1 also shows structural commonality with SH3BP4, which is located at the plasma membrane and regulates the clathrin-dependent internalization of the transferrin receptor [17]. Based on this commonality, as well as the changing the cellular transcription characteristic of MACC1, it can be assumed that MACC1 plays an important role in signaling pathways by inducing receptor-based signaling leading to transcriptional activation [18] [19].

Taking a look at MACC1 expression in malignant tissues, apparently high levels of expression were observed in tumours of patients who had multiple separate occurrences of metastases. This again suggests that MACC1 is an important prognostic biomarker for metastasis development [20]. Remarkably, MACC1 expression did not occur at higher levels in colon adenomas compared with normal tissues; therefore, MACC1 expression correlates with formation of benign into malignant tissues and exhibits the metastatic potential of a tumour [21] [22].

Recent reports of transgenic mouse models for MACC1 have shown its ability to induce tumour progression characteristic by expression of the pluripotency markers, Nanog and Oct4. Based on this, MACC1 shows promise as a novel prognostic biomarker for the metastasis of colorectal cancer and the survival of patients [23]. It is also shown that MACC1 associates with pathways in CSC. Because CSC are the main contributors to tumour progression and metastasis, this provides a new therapeutic area for MACC1 [24].

Conclusion

Our results show a two-fold increase in MACC1 gene expression in CSC compared with cancer cell in head and neck cancer, which indicates that MACC1 gene has the potential to be a therapeutic target. Further investigation and analysis is warranted.

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