MAY METALLIC BIOMATERIALS USED FOR ORTHOPEDIC IMPLANTS PROMOTE CANCER PROCESSES? PRELIMINARY TRASRIPTOMICS RESEARCH ON HUMAN ARTICULAR CHONDROCYTES.

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Introduction

There are numerous reports about the formation of cancer changes adjacent to the implant or in places distant but temporally correlated with the implantation. This phenomenon is strongly marked in dental implantology, where one of the main types of cancer located in close proximity of dental implants is squamous cell carcinoma [1]. At the moment there is no indisputable data on the initiating of carcinogenesis by implants used in orthopedics, although this subject has been often discussed in works from the last three decades. For example, after total hip arthroplasty, the appearance of malignant neoplasms in the area of endoprostheses, including osteoma, osteosarcoma, lymphoma or squamous cell carcinoma has been reported [2,3]. However, no mechanism is fully confirmed, and the issue of accelerated tumour induction at the implantation site is still poorly understood and unclear. In the light of above information, our project assumes verification whether immortalized cell lines with neoplastic phenotype show an altered response to contact with implant material compared to primary cell lines of the same type.
In this report, the results from analysis of changes in expression of cancer-related genes in the human articular chondrocytes, performed by the use of qRT-PCR technique, have been presented.

Materials and Methods

The study was carried out for three types of materials used for implant production (medical steel AISI 316L, titanium alloys Ti6Al4V and Ti6Al7Nb). On the basis of the previously prepared literature review, 19 genes promoting cancer formation were selected and a custom PCR plate was designed. The primary chondrocytes of the HC-a line were purchased from ScienCell Research Laboratories (cat no. 4650). Six independent RNA isolation experiments from HC-a cells grown on the surfaces of the tested biomaterials were carried out using GeneMATRIX Universal RNA Purification Kit (EURx Ltd). The cells grown on the surface of a standard culture flask was used as a control.
Then, using Agilent's 2100 bioanalyzer, the quality and purity of isolated RNA was assessed. After this, with the use of the iScript cDNA Synthesis Kit (BIO-RAD), the reverse transcription was carried out.
The key stage of this study was to perform six qRT-PCR reactions using the CFX96 Touch thermal cycler (BIO-RAD) and 2xSsoAdvanced Universal SYBR Green Supermix reagent (BIO-RAD) on 96-well custom plates containing lyophilisates of 19 genes associated with development of tumorigenic processes.

Results and Discussion

The starting point for analysis of changes in gene expression in qRT-PCR is the Cq value, which is a measure of gene expression and can be defined as the number of cycles after which the signal exceeds the detection threshold. Differential analysis was performed, i.e. the expression of a examined gene in the tested sample (RNA from cells grown on the surface of biomaterials) was referred to the expression of this gene in the control sample (RNA from cells cultured without contact with the materials). The obtained results are presented as so-called ratio or fold change (Fc), i.e. the ratio of the Cq value of the sample to the Cq of the control. GAPDH and ACTB genes were selected as the reference genes.
The nonparametric one-way ANOVA test used for statistical analysis of results (statistical significance $p < 0.05$) indicated the occurrence of statistically significant changes of the average values of Fc for analyzed genes in the chondrocytes grown on the examined surfaces (AISI 316L, Ti6Al4V and Ti6Al7Nb) in comparison to control cells cultured without contact with any biomaterial. However, for a full interpretation of the results it is necessary to continue further studies including the analysis of changes in the expression of selected genes in chondrosarcoma cells (chondrocytes with neoplastic phenotype).

Conclusions

Analysis of changes in the expression of cancer-related genes plays a vital role in the assessment of risk of induction or intensification of carcinogenesis by the implants used in orthopedics. Compilation of qRT-PCR experiments carried out on primary and cancer cells in parallel will allow to identify possible future contraindications for patients with a genetic predisposition to cancer or with cancer history. What is more, this approach may be a crucial step in the selection of the right biomaterial for a specific patient.

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