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Genome-wide transcriptome induced by nickel in human monocytes

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ABSTRACT

Nickel-containing alloys are frequently used in the biomedical field, although, owing to corrosive processes metal ion leaching is inevitable. Due to nickel ion (Ni^{2+}) leaching several adverse effects are described in the literature. However, only a few studies evaluated the genetic profile of Ni^{2+} in human cells which is of great importance since nickel-induced effects differ between humans and mice as a result of species-specific receptor variability.

Thus, we investigated gene expression induced by Ni^{2+}in human monocytes using a transcriptome-wide approach determining new target genes implicated in nickel-induced pathologies. Monocytes were isolated from healthy volunteers of Central European origin using stringent inclusion criteria. Cells were challenged with different Ni^{2+} concentrations. Array-based gene expression analysis was performed comprising more than 47,000 transcripts followed by pathway analyses. Transcriptional data were validated by protein and cell surface markers.

Ni^{2+} significantly influenced the expression of 1385 transcripts in a dose-dependent manner. Apart from known targets (CCL20↑, PTGS2↑, MTs↑, SLCs↑), we identified new candidates implicated in Ni^{2+}-elicited processes (various microRNAs↑, INSIG1↑, NAMPT↑, MS4A6A↓, DHRS9↓). Several of these transcripts correspond to immunity, inflammation and were shown to be involved in cellular reactions related to hypersensitivity, cancer, colitis, and encephalitis. Moreover, 459 canonical pathways/signaling, 500 pathologies and 2687 upstream regulators were detected. Protein results validated our findings.

To our knowledge, the present systematic transcriptome-wide expression study is the first which explored Ni^{2+}-elicited cell responses in human primary monocytes identifying new target genes, pathways and upstream regulators of relevance to diagnostic and therapeutic strategies.

Statement of Significance

Nickel is widely applied in the biomedical field, although several adverse effects are documented in the literature due to nickel ion (Ni^{2+}) leaching. In humans, allergic reactions like contact dermatitis are the most common adverse effect to Ni^{2+}, whereas serious concerns relate to possible systemic and carcinogenic activities. Using a systematic genome-wide transcriptional approach in human primary monocytes unveil new target genes, pathways and upstream regulators implicated in nickel-elicited immune response which are of significance to diagnostic and therapeutic strategies. This approach provides new information of how host-derived immune response contributes to the interaction with antigens and supports the interplay between metal ions and systemic diseases.

1. Introduction

Despite the fact that corrosive processes lead to metal ion leaching, Nickel-containing alloys are still frequently used in the biomedical field. As a consequence, in vitro studies have

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investigated the interaction of nickel ions (Ni^{2+}) with tissue components demonstrating that they may induce allergic, cytotoxic, mutagenic or even carcinogenic effects [1–4]. In humans, allergic reactions like contact dermatitis are the most common adverse effect to Ni^{2+}, whereas serious concerns relate to possible systemic and carcinogenic activities. Epidemiological studies have shown that chronic exposure to nickel-containing dust was associated with an increased risk of malignant tumors [3,5].

Some of these adverse effects may arise from binding to a specific immune cell receptor (Toll like receptor (TLR)4), others may be attributed to the activation of the hypoxia-inducible factor (HIF) complex [6,7]. Thereby, it is assumed that divalent cations like Ni^{2+} inhibit proteosomal degradation of HIF-1α which consequently translocates into the nucleus and dimerizes with HIF-1β activating targeted mediators involved in angiogenesis, cell metabolism, immune cell migration and apoptosis [7]. Notably, the HIF complex is implicated in tumorigenesis and control the innate and adaptive immunity [8].

TLR4 is a conserved receptor on cell membranes and expressed on several immune cells including monocytes. TLR4-induced signaling causes an inflammatory reaction via activation of specific transcription factors such as nuclear factor (NF-)κB. After nuclear translocation, NF-κB initiates the expression of various target genes responsible for proliferation, differentiation, inflammatory processes, apoptosis, or stress responses [9].

To decipher cellular mechanisms induced by biomaterials, microarray technology has emerged and successfully performed in different cell types [4,10–12]. However, only a few studies evaluated the genetic profile of Ni^{2+} in human primary cells. Schmidt et al. revealed that Ni^{2+} binding to TLR4 is species-specific and mouse TLR4 is not able to generate the nickel-induced TLR4 response [6]. The authors hypothesized that this response is an important mechanism in contact hypersensitivity, the molecular basis for the prevalent contact dermatitis. Since human monocytes play a critical part in the systemic immune defense and can differentiate into macrophages or dendritic cells (DCs) which are highly implicated in allergic reactions and tumorigenesis, the present study focused on the transcriptome induced by Ni^{2+} in these cells [13,14]. Therefore, more than 47,000 transcripts were analyzed to provide transcriptome-wide expression profiles of well-characterized genes, gene candidates and splice variants derived from the National Center for Biotechnology Information Reference Sequence. Our findings expand the understanding of nickel-elicited responses, and identify new target genes and pathways of relevance to diagnostic and therapeutic strategies in contact dermatitis and carcinogenesis.

2. Materials and methods

2.1. Volunteer recruitment and monocyte isolation

The study was approved by the ethics committee of the University of Bonn (Nr. 352/13), and all subjects provided written informed consent prior to inclusion. Fourteen male volunteers of Central European origin (18–35 years) were recruited, and peripheral blood samples were obtained. All fourteen donors were healthy which was validated by a questionnaire including the question of known allergies and in specific nickel hypersensitivity. Females were excluded, since hormonal factors may influence transcriptional expression patterns. The following inclusion criteria were applied: non-smoker; no acute or chronic infection; no vaccination within the four weeks prior to blood sampling; C-reactive protein levels <2.5 mg/dl; monocyte purity >95%; and monocyte overnight survival >85%. On the basis of these criteria, four individuals were excluded. The cells of the remaining ten individuals underwent further processing.

As previously described [15], peripheral blood mononuclear cells from 100 ml whole blood (PBMC) were purified using a Ficoll-Plaque density gradient. $CD14^+$ monocytes were then separated by magnetic-activated cell sorting and CD14-microbeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Cell purity was determined by flow cytometry followed by resuspension of cells in RPMI 1640 GlutaMAX™ supplemented with 10% heat-inactivated FBS (all from Gibco®, Life Technologies, Waltham, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (PAA Laboratories/GE Healthcare, Pasching, Austria).

2.2. Monocyte treatment

Monocytes were cultured overnight in 96-well round bottom wells at a density of 500,000 cells/well in 100 µl. Cell survival was analyzed optically using trypan blue staining, followed by Annexin V (Apoptosis Detection Kit) and propidium iodide detection (eBioscience, San Diego, USA) via flow cytometry. According to previous in vitro studies [4,6], different nickel(II)-chloride-hexahydrate (Ni^{2+}) (Sigma-Aldrich, Taufkirchen, Germany) concentrations were chosen (1-10-100-1000-10,000 µmol/l) and tested for cell viability after 72 h using XTT assays (PromoKine, Heidelberg, Germany). Afterwards, the lethal dose for human monocytes was calculated (LD50 = 797.73 µmol/l) and cells were treated with different nickel concentrations (200 and 400 µmol/l) which did not affect cell viability. In order to prevent non-specific cell stimulation via TLR4 activation [6], Ni^{2+} solution had been tested for endotoxin contamination prior to use. Non-stimulated monocytes served as controls.

After stimulation, supernatants were collected, cells were lysed in RLT Plus buffer (Qiagen, Venlo, Netherlands) and stored at −80 °C until analysis.

2.3. RNA extraction

RNA was extracted from lysed monocytes using the Qiagen All-Prep DNA/RNA Mini Kit in accordance with the manufacturer's instructions. For quality control purposes, RNA concentrations were measured using NanoDrop (PeqLab, Erlangen, Germany) and RNA degradation was measured using Bioanalyzer (Agilent Technologies, Santa Clara, USA).

2.4. Transcriptome analysis

RNA was amplified and biotinylated using the Illumina Total-Prep RNA Amplification Kit (Life Technologies). Subsequent array-based gene expression analysis was performed using the Human HT-12 v4 Expression BeadChip (Illumina, San Diengo, USA) and the automated protocol, in accordance with the manufacturer's manual. The HT-12 v4 Expression BeadChip comprises 47,231 probes. The arrays were scanned with an iScan microarray scanner (Illumina, San Diego, CA, USA). Statistical analysis was performed using functions implemented in the statistical software packages R and Bioconductor. All data were subjected to quantile-normalization using the limma package. Selection of differentially expressed genes was performed using the following filter criteria: fold change (FC) $\geqslant$ 1.7 and $P < 0.05$. The FC is the ratio of the group mean values. Transcripts with at least a 1.7-FC were selected. All adjusted p-values P were calculated using a Student's t-test, and adjusted for multiple testing using Benjamini-Hochberg correction.

2.5. Protein analysis

To determine the impact of identified transcripts, ELISAs or Multiplex Immunoassays (eBioscience or R&D, Minneapolis, USA)