

Original Article

Transcriptional profiling defines the effects of nickel in human epidermal keratinocytes

Alix Gazel¹, Martin Rosdy², Carine Tornier², Anne De Brugerolle De Fraissinette², Miroslav Blumenberg^{1 3 4 *}

¹Department of Dermatology, New York University School of Medicine, New York, New York

²SkinEthic Laboratories, Palmeira Building, Nice, France

³Department of Biochemistry, New York University School of Medicine, New York, New York

⁴NYU Cancer Institute, New York University School of Medicine, New York, New York

email: Miroslav Blumenberg (blumem01@med.nyu.edu)

*Correspondence to Miroslav Blumenberg, Department of Dermatology, NYU School of Medicine, 550 First Ave., New York, NY 10016.

Abstract

Nickel is a ubiquitous and virtually unavoidable environmental pollutant and occupational hazard, but its molecular and cellular effects are not well understood. Human epidermal keratinocytes are the sentinel and the primary target for nickel. We treated with nickel salts skin equivalents containing differentiating epidermal keratinocytes grown on air-liquid interface in standard cell culture conditions. We identified the transcriptional profiles affected by nickel in reconstructed human epidermis (RHE) using DNA microarrays. The Ni-regulated genes were determined at two time points, immediate-early, 30 min after treatment, and late, at 6 h. Using in silico data analysis, we determined that 134 genes are regulated by nickel; of these, 97 are induced and 37 suppressed. Functional categories of regulated genes suggest that Ni inhibits apoptosis, promotes cell cycle and induces synthesis of extracellular matrix proteins and extracellular proteases. Importantly, Ni also regulates a set of secreted signaling proteins, inducing VEGF, amphiregulin, PGF, GDF15, and BST2, while suppressing IL-18, galectin-3, and LITAF. These secreted proteins may be important in Ni-caused allergic reactions. Ni induced inhibitors of the NF κ B signaling pathway, and suppressed its activators. Correspondingly, NF κ B binding sites were found to be overrepresented in the Ni-suppressed genes, whereas cFOS/AP1 binding sites were common in the Ni-induced genes. Significant parallels were found between the Ni-regulated genes and the genes regulated by TGF β , EGF, glucocorticoids, or Oncostatin-M. The comprehensive identification of Ni-regulated genes in human epidermal equivalents significantly advances our understanding of the molecular effects of nickel in skin. *J. Cell. Physiol.* © 2008 Wiley-Liss, Inc.

Received: 28 March 2008; Accepted: 16 June 2008

Digital Object Identifier (DOI)

10.1002/jcp.21536 [About DOI](#)

Article Text

The carcinogenic and allergenic effects of metallic nickel and its salts have been known for a long time, over a century (Blaschko, [1889]). Nickel is both a well recognized occupational carcinogen and one of the most common allergens, second only to poison ivy/poison oak as the cause of allergic contact dermatitis in US (Kasprzak et al., [2003b]; Costa et al., [2005]; Durham and Snow, [2006]). The North American Contact Dermatitis Data Group surveys show that 16.7% of the population have allergy to nickel, and this incidence seems to be increasing (Krob et al., [2004]; Pratt et al., [2004]). Nickel is virtually ubiquitous and essentially unavoidable in daily life: it is found in coins, jewelry, zippers, buttons, all stainless steel products etc. (Nestle et al., [2002]). The ear and body piercing vogue has triggered an epidemic of allergy to nickel. Nickel is also an

occupational hazard for workers in nickel production, purification and especially electroplating, as well as toll collectors, cleaning crews, musicians, hospital staff etc. Nickel allergy presents as a skin rash caused by contact with nickel-containing objects. Like other allergies, nickel allergy is acquired, usually after prolonged exposure. It is more common in women, but the incidence in men is increasing because of the popular trend in body piercing. Nickel can also be found in certain foods, particularly in legumes, grains, and fish. Nickel allergy may develop at any age and persists for many years, often for a lifetime. At the moment there is no therapeutic approach to desensitize a person with nickel allergy and avoiding skin contact is the only effective prevention of nickel caused rashes. Usually symptoms clear up once the contact with nickel is prevented and a cortisone cream or lotion is applied topically. Particularly insidious aspect of this problem is the essentially unavoidable, ubiquitous presence of nickel in our everyday lives like in coins, which we handle all the time (Nestle et al., [2002]). While nickel allergies constitute a widespread and serious health hazard, the molecular effects of nickel in the epidermis have not been systematically studied.

As a carcinogen, nickel works in lung cells through mechanisms involving epigenetic gene silencing and hypoxia signaling pathways (Costa et al., [2005]). The nickel-induced carcinogenic mechanisms range from ascorbate depletion, histone modification, chromatin damage and reorganization, production of reactive oxygen species and paradoxically, induction of hypoxia-inducible transcription factor HIF-1, as well as prevention of apoptosis, perhaps via the activation of the NF κ B pathway (Reid et al., [1994]; Chen et al., [2001]; Zoroddu et al., [2002]; Costa et al., [2003]; Salnikow et al., [2003]; Kasprzak et al., [2003a]; Salnikow and Kasprzak, [2005]; Ding et al., [2006]; Karaczyn et al., [2006]). Notwithstanding the extensive studies of nickel-induced carcinogenesis, very little is actually known about the signal transduction pathways responsive to nickel: NF κ B and HIF-1 are the only two nickel-responding transcription factors identified. It is not known whether the same signal transduction mechanisms may be involved in the epidermis. Bioinformatics of nickel action is in its infancy: to date, only three studies used DNA microarrays to examine the transcriptional effects of nickel (Cheng et al., [2003]; Hirota and Moro, [2006]; Schoeters et al., [2006]).

Keratinocytes are the primary target of nickel and initiate both the antigen presenting immune responses and the proinflammatory signals in nickel allergies. Unfortunately, very little is known about the molecular effects of nickel in keratinocytes (Nestle et al., [2002]; Garner, [2004]; Krob et al., [2004]; Pratt et al., [2004]). While the nickel-responsiveness in various T cells received significant attention (Sebastiani et al., [2002]; Cavani et al., [2003]; Minang et al., [2006]), the molecular effects of nickel on the epidermal keratinocytes, the cells that are in the primary contact with nickel, have not been extensively studied. It was shown that nickel can induce in keratinocytes the production of cytokines TNF α , IL-1, and IL-6 (Oxholm et al., [1991]; Gueniche et al., [1994]; Lisby et al., [1995]). Nickel also induces keratinocyte proliferation and KGFR expression (Marchese et al., [2003]). Importantly, nickel induces the expression of CD80 (a.k.a. B7), an essential co-stimulatory protein on antigen presenting cells (Wakem et al., [2000]). This suggests that keratinocytes, nonprofessional antigen presenting cells, are the initiator of allergic contact sensitivity reaction to nickel. The antigen-presenting role of keratinocytes in nickel allergies remains virtually unexplored.

Our goal is to elucidate the molecular events triggered by nickel in human epidermal keratinocytes. We decided to explore the transcriptional effects of NiSO₄ in epidermal keratinocytes using

Affymetrix DNA microarrays. We used reconstructed human epidermis (RHE) rather than cultured keratinocytes or human skin because, on one hand, keratinocyte monolayers cannot differentiate in culture and to that extent do not faithfully represent the three-dimensional differentiation epidermis (cf. Finelt et al., [2005]; Gazel et al., [2006b]), and on the other hand, we have found that the responses of human skin from donors can be quite variable, which requires multiple samples to obtain reliable data (Gazel et al., [2003]). We also decided to examine two time points, 30 min and

6 h after the treatment with nickel. The first one aims to identify the “immediate-early” response genes, which may include the signal transduction proteins and transcription factors directly responsible to nickel treatment. The 6 h point could also include the genes regulated by the “immediate-early” genes. We chose NiSO_4 , the standard source of soluble nickel, at the relatively high concentration, which was determined in preliminary experiments to having a profound effect on the skin equivalents. We chose these conditions to ensure a clear, robust and unambiguous response by the keratinocytes.

We found that nickel regulates 134 genes, 97 are induced, and 37 suppressed. Nickel suppresses apoptosis-related genes, while promoting cell cycle and the remodeling of the extracellular matrix. Importantly, nickel also regulates a set of secreted signaling proteins, which may be important in nickel-caused allergic reactions. cFOS/AP1 binding sites were found to be overrepresented in the nickel-induced genes, while $\text{NF-}\kappa\text{B}$ sites were numerous in the nickel-suppressed ones. The set of nickel-regulated genes significantly overlaps the sets regulated by $\text{TGF}\beta$, EGF, glucocorticoids or Oncostatin-M.

Materials and Methods

Production and maintenance of skin equivalents

The reconstructed human epidermis, RHE, (SkinEthic Laboratories, Nice, France) consists of a fully differentiated three-dimensional multi-layered keratinocyte structure grown on the air-liquid interface at 37°C , 5% CO_2 and saturated humidity for 17 days in modified, chemically defined medium MCDB 153, supplemented with 1 ng/ml EGF, 0.4 $\mu\text{g/ml}$ hydrocortisone, and 5 $\mu\text{g/ml}$ insulin (growth medium) (Rosdy and Clauss, [1990]; Rosdy et al., [1997]; Bernard et al., [2000]; Tornier et al., [2006]; Gazel et al., [2003], [2006b]). All cells were expanded and treated according to the SkinEthic quality-controlled production procedures according to SkinEthic institutional approval.

NiSO_4 tissue treatment

At day 17.4 cm^2 RHE samples were incubated in 1 ml of maintenance medium in six-well plates. Untreated tissues and 0.05% sodium dodecyl sulfate (SDS) treated tissues (120 μl) were used as controls. To treated cultures, we added 120 μl of NiSO_4 , 1.7 mg/ml diluted in 0.05% SDS solution, for the final concentration of 11 μM (Sigma-Aldrich, Saint Quentin Fallavier, France). NiSO_4 was applied topically onto each tissue. The NiSO_4 and 0.05% SDS solutions were spread homogeneously onto the whole surface of the stratum corneum using a 4 cm^2 piece of sterile gauze. Duplicate tissues were incubated for 30 min or for 6 h, at 37°C , 5% CO_2 . The treated and untreated, control samples were harvested for microarray analysis, immunohistochemical studies, Western blotting, and quantitative real-time RT-PCR analysis. We chose this treatment because it allowed us to harvest all samples on the same day, that is, at the exactly the same stage of growth, differentiation and cornification. The untreated tissues were used as negative controls. The concentration chosen, 1.7 mg/ml NiSO_4 , 11 μM , was guided by our preliminary experiments and the experiments described in the literature (Cheng et al., [2003]).

Preparation and hybridization of probes

We isolated the total RNA from treated and untreated keratinocytes using RNeasy kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. The inserts from artificial skin substitutes were first detached in 4 ml dispase II (Roche, Meylan, France) in a 100 mm Petri dish at room temperature. After 3 min, the epidermal tissues were removed from the polycarbonate filter inserts with fine RNase-free forceps and further processed. All solutions contained RNase inhibitors; skin is particularly rich in RNases, necessitating their inhibition. To ensure good RNA

quality, 28S and 18S ribosomal bands were visualized on a non-denaturing agarose gel and OD_{260/280} spectrophotometric ratio of at least 1.8 is ascertained. Approximately 5 µg of total RNA was reverse-transcribed, amplified, and labeled as described (Li et al., [2001]; Banno et al., [2004c]; Gazel et al., [2006b]). Fifteen micrograms of labeled cRNA was fragmented and hybridized to HGU95Av2 arrays (Affymetrix, Santa Clara, CA), with the capacity to display transcript levels of approximately 12,000 human genes. Arrays were washed, stained with anti-biotin streptavidin-phycoerythrin labeled antibody and scanned using the Gene Array Scanner system (Hewlett-Packard, Palo Alto, CA), and GeneChip 3.0 software (Affymetrix) to determine the expression of each gene, as described by Affymetrix.

Gene array data analysis

Generally, we used the same data analysis approach as described in our previous publications (Li et al., [2001]; Banno et al., [2003], [2004b],[c], [2005]; Finelt et al., [2005]; Molenda et al., [2006]; Radoja et al., [2006]; Gazel et al., [2006a],[b]). Intensity values from the chips were obtained using Microarray Suite version 5.0 (Affymetrix), and scaled by calculating the overall signal for each array. To compare data from multiple arrays, the signal of each probe array was scaled to the same target intensity value of 500 arbitrary units. To eliminate genes exhibiting potential false positive differential expression, first we calculated the median intensity for all genes scored as “absent” on the chips and the standard deviation of this intensity; then we select for further analysis only genes that possessed relative signal intensity greater than one standard deviation above that median. We included in the analysis only those genes determined by the Affymetrix algorithm to be present in at least one sample. The eliminated genes are not expressed in keratinocytes, or expressed at such low level that their measurements are unreliable. This selection usually eliminates approximately half of the genes represented on the chips. To improve reliability even more, we checked individually the absolute expression levels and detection *P* values at all time points. Differential expressions of transcripts at each time point is determined two ways: using the standard discrimination evaluation GeneChip data mining software Affymetrix-DMT3.0 and using the RMAExpress set of programs to select genes with a twofold or better difference in expression (Bolstad et al., [2003]). Thus, we combined two different and stringent selection criteria, one requiring a statistically significant difference in signal intensity, the second a significant fold change, which ensures that the genes we list as “regulated” are reliably affected by our experimental treatment. Affymetrix' GCOS programs was used to derive scatter-plots, perform the *t*-test and the Mann-Whitney test, and calculate fold change in preliminary data analyses.

The regulated genes were functionally classified according to an extensive gene annotation table describing the molecular function and biological category of the genes present on the chip (Banno et al., [2003], [2004a], [2005]). The table is based on the Gene Ontology Consortium <http://cgap.nci.nih.gov/genes/gobrowser>. In addition, we used L2L program to identify biological processes and cell components statistically over-represented in our lists of differentially expressed genes (Newman and Weiner, [2005]). We used the 2005-06-15 version. The lists of regulated genes were compared with the gene ontology categories of *Biological Process*, *Molecular Function* and *Cellular Component*, as well as with a set of lists of regulated genes in the L2L database. These analyses followed the established patterns we have used to identify the biological processes and signaling pathways over-represented among the genes regulated by Interferon-γ, TNFα, IL-1, glucocorticoids etc., as published (Banno et al., [2005]; Molenda et al., [2006]; Radoja et al., [2006]; Gazel et al., [2006a],[b]; Stojadinovic et al., [2007]).

To identify the transcription factors responsive to NiSO₄ signaling, we used the oPOSSUM set of microarray data analysis programs. The oPOSSUM program uses weighted recognition site matrices of transcription factor binding sites to analyze the homologous sequences of the human and murine genomes (Ho Sui et al., [2005]). Given a list of genes induced by NiSO₄, the program first identified the genes for which both the human and murine sequences are available, and are homologous above a certain threshold. Presumably, the homologous sequences have been

conserved through evolution and the transcription factor binding sites within them are more relevant than in random, un-conserved sequences. We used the 70% sequence conservation threshold. Next, the program identifies the transcription factor binding sites in the retained sequences, and finally calculates the statistical probability of finding that many sites, relative to the number of sites in a collection of ~7,000 randomly selected genes.

We used the LOLA programs for comparing lists of genes (Cahan et al., [2005]). LOLA program compared the lists of NiSO₄-regulated genes with the lists of genes regulated by various treatments in our extensive databank (Li et al., [2001]; Banno et al., [2003], [2004b],[c], [2005]; Finelt et al., [2005]; Molenda et al., [2006]; Radoja et al., [2006]; Gazel et al., [2006a],[b]).

The raw data from the experiments presented in this work are deposited with the GEO, <http://www.ncbi.nlm.nih.gov/geo/>, under the access number (pending).

Results



We compared the transcription profiles of the two NiSO₄-treated and two control RHEs at 30 min and 6 h after addition of the salt. The results of the microarray studies are highly interesting and illuminating. In Table 1 we list the 134 genes identified as regulated by nickel in these experiments. The regulation of gene expression is given in the log₂ units, which means +1 is a twofold induction, -1 is a twofold suppressed etc.

Table 1. Nickel-regulated genes in skin equivalents 30 min and 6 h after treatment

BST2 are induced. These play very important roles in angiogenesis, maturation of B-cells and cell proliferation. Their roles in nickel-induced allergies and inflammation will be the subject of our future studies. In contrast, IL-18, LGALS3, and LITAF are suppressed. IL18 can induce interferon production and is associated with coronary disease, but it apparently inhibits the activation of CD4 cells (Van Der Sluijs et al., [2005]). Thus, the suppression of IL18 production may be another pro-inflammatory effect of nickel. LGALS3 (a.k.a. galectin-3) plays a role in re-epithelialization during wound healing and interacts with macrophages. LITAF stimulates monocytes and macrophages, regulating the activity of TNF α . The specific roles of LGALS3 and LITAF in nickel responses are currently unexplored.

The potential effects of nickel on the remodeling of the extracellular matrix are one of the completely novel insights of these experiments. Specifically, nickel induced the expression of extracellular proteases kallikrein 10, prostasin and MMP9 (a.k.a. gelatinase B). Moreover, nickel suppressed the expression of protease inhibitors SerpinB3 and SerpinB4 (squamous cell carcinoma antigens 1 and 2). Importantly, these Serpins are known to inhibit protease Cathepsin S, which plays a role not only in remodeling of the extracellular matrix (Serveau-Avesque et al., [2006]), but also in antigen processing and presentation (Schwarz et al., [2002]; Wiendl et al., [2003]). In addition, nickel induced the expression of extracellular matrix proteins, including syndecan-4, collagen-16 α 1, and ECM1. In aggregate, these results point to a previously unknown effects of nickel on the extracellular matrix.

We used two sets of microarray analysis programs, L2L and DAVID (Dennis et al., [2003]; Newman and Weiner, [2005]) to analyze the ontological categories over-represented in the nickel-regulated genes. L2L output is presented in Table 2. Interestingly, the nickel regulated processes include members of the NF κ B cascade among both the induced and the suppressed genes. However, while all three suppressed genes are activators of the cascade, TNFAIP3, an induced gene, is a known strong suppressor. The effects of ECM1, BST2 and FLNA on the NF κ B pathway are indirect. From these data we conclude that nickel suppresses the NF κ B signal transduction pathway. This completely novel and unexpected finding could lead to new treatment modalities for nickel allergies.

Table 2. Significant ontological categories and NF κ B cascade genes regulated by nickel

Induced by nickel					Suppressed by nickel				
ListName	Genes	Expected	Actual	Fold p-value	ListName	Genes	Expected	Actual	Fold p-value
Process					Process				
regulation of I κ B/NF κ B cascade	68	0.72	4	5.6 5.87E-03	positive regulation of I κ B/NF κ B cascade	65	0.26	3	12 2.35E-03
regulation of signal transduction	79	0.83	4	4.8 9.89E-03	regulation of I κ B/NF κ B cascade	68	0.27	3	11 2.67E-03
					regulation of signal transduction	79	0.32	3	9.5 4.08E-03
					I κ B/NF κ B cascade	85	0.34	3	8.8 5.01E-03
					regulation of proteolysis	2	0.01	1	124 8.02E-03
Function					Function				
amino acid permease activity	10	0.11	2	19 4.72E-03	amino acid permease activity	78	0.31	4	13 2.93E-04
neutral amino acid transporter	11	0.12	2	17 5.73E-03	neutral amino acid transporter	78	0.31	4	13 2.93E-04
						18	0.07	2	28 2.37E-03
						156	0.63	4	6.4 3.81E-03
Genes involved in the I κ B/NF κ B cascade									
TNFAIP3 (inhibitor!)					LITAF				
ECM1					TRIM38				
BST2					TNFSF10				
FLNA									

Biological processes and molecular functions with P -value 9.9×10^{-3} or better were selected. Note that “regulation of NF κ B kinase” was flagged in both the induced and suppressed processes. Therefore, we identified individually all the genes in these two categories (bottom box).

The suppression of proteolysis inhibitors is another novel and unexpected aspect of nickel action. Proteolysis, in the form of extracellular matrix degradation and release of cytokines from their inert precursors, is an important aspect of inflammatory responses, which we propose to study in the future.

We note in passing that several amino acid transport proteins are induced by nickel.

Using the oPOSSUM programs (Ho Sui et al., [2005]), we searched for the transcription factors that potentially effect the nickel-mediated transcriptional changes. These are listed in Table 3. The binding sites for c-FOS and SP1 are over-represented among the nickel-induced genes; these transcription factors may be activated by the nickel treatment. As expected from the above analysis (Table 2), the NF κ B sites are over-represented in the genes suppressed by nickel (Table 3). The two completely independent lines of data analysis produced congruent results regarding the role of NF κ B in responses to nickel, which we find especially satisfying.

Table 3. Transcription factors with binding sites over-represented in the nickel-regulated genes

One-tailed Fisher Exact Probability Analysis											
Selected parameters:											
Conservation level:		Top 30%		Upstream length:		5000bp					
Matrix match score:		85%		Downsream length:		5000bp					
Genes submitted					167	Genes submitted					95
Genes included					113	Genes included					59
Genes excluded					54	Genes excluded					36
Induced by Ni					Suppressed by Ni						
TF	Hits	non-hits	Z-score	p-value	TF	Hits	non-hits	Z-score	p-value		
c-FOS	70	43	14.82	7.02E-06	ARNT	21	38	15.56	2.46E-04		
ARNT	37	76	14.00	1.31E-05	n-MYC	40	19	10.35	1.17E-03		
SP1	60	53	21.64	4.25E-05	Ahr-ARNT	37	22	8.09	1.45E-03		
MZF 1-4	97	16	19.50	2.44E-04	c-REL NFκB	16	43	13.12	2.67E-03		
n-MYC	71	42	13.52	6.44E-04	Max	18	41	8.27	4.79E-03		

The top five binding sites are presented in the induced and suppressed sets. The prominent transcription factors discussed in the text are marked with red ovals. Note that the sites for the Myc family of factors, n-Myc, Max, and Arnt, were found over-represented in both the induced and the suppressed genes.

Interestingly, ARNT and n-MYC binding sites are also present both in the induced and in the suppressed genes (Table 3). ARNT is required for the translocation of the ligand-bound dioxin receptor (a.k.a. Ah receptor) to the nucleus, causing the induction of cytochromes and other enzymes that participate in metabolism of xenobiotics. ARNT is a common dimerization partner of Hypoxia-inducible factors-1 and -2 (HIF1, HIF2), transcription factors that play essential roles in cellular and systemic homeostatic responses to hypoxia (Wang and Semenza, [1995]; Hogenesch et al., [1997]). Hypoxia is a known effect of nickel toxicity (Maxwell and Salnikow, [2004]; Costa et al., [2005]). These results give tantalizing early glimpses into the molecular signaling by nickel in keratinocytes.

c-MYC is an oncogene transcription factor that regulates epidermal stem cell recruitment and differentiation (Gandarillas and Watt, [1997]; Waikel et al., [1999]). We find MYC binding sites in both the induced and suppressed genes. Interestingly, a connection between these effects of nickel and MYC has been reported before, but not examined in great molecular detail (Zhou et al., [1998]; Shimono et al., [1999]; Costa et al., [2005]; Kowara et al., [2005]).

The large database of microarray experiments we performed with human epidermal keratinocytes provides a superb background data set that can be easily analyzed in the context of a new approach. Using the LOLA set of programs (Cahan et al., [2005]), we compared the nickel-regulated sets of genes with the sets identified in our previous experiments. The comparison identified a nexus between the TGF β -regulated and Ni-regulated genes shown in Table 4 (the TGF β data are from J. Zavadil and M. Blumenberg, in preparation). Statistically highly significant overlaps exist both in the induced and the suppressed sets. The EGF- and dexametasone-regulated genes (Stojadinovic et al., [2006]) also overlap with the nickel-regulated ones. Potentially as important is the inverse correlation with the Interferon- γ and Oncostatin-M regulated genes: many of the genes induced by nickel are suppressed by these two cytokines, while the genes suppressed by nickel are induced by OsM (overlap with the interferon-induced genes did not reach statistical significance; we use P -value 10^{-5} as cut-off, Table 4). The identification of the genes regulated in common by nickel and various growth factors and cytokines (Banno et al., [2003], [2004c], [2005]; Finelt et al., [2005]; Haider et al., [2006]; Molenda et al., [2006]), as well as genes shown to be regulated by epidermal differentiation and specific signal transduction pathways (Blumenberg, [2005]; Radoja et al., [2006]; Gazel et al., [2006a]) will greatly augment our understanding of the molecular effects of nickel in human epidermis.

Table 4. Significant commonalities between the nickel-regulated genes and other categories in our databank

Selected genes		Intersect	P-Value
Ni-UP 174	TGF β -UP 643	43	1.73E-32
Ni-UP 174	EGF-UP 195	23	3.65E-23
Ni-UP 174	DEX-UP 126	16	5.86E-17
Ni-UP 174	IFN γ -DN 262	19	2.50E-15
Ni-UP 174	OsM-DN 175	16	1.16E-14
Ni-DN 100	DEX-DN 252	14	2.29E-13
Ni-DN 100	EGF-DN 209	10	3.07E-09
Ni-DN 100	TGF β -DN 451	13	5.63E-09
Ni-DN 100	OsM-UP 280	8	6.01E-06
Ni-DN 100	IFN γ -UP 244	5	1.60E-03

There is a significant overlap between the nickel-regulated genes and the TGF β -, EGF-, and Glucocorticoids-regulated genes (DEX). There is a significant “inverse” overlap with the interferon- γ - and OsM-regulated genes (nickel-induced genes overlap the OsM-suppressed ones and vice versa).

Discussion



Large scale transcriptional profiling of epidermal equivalents, comprised of keratinocytes differentiating from basal through cornified layers, promises to uncover new and important molecular processes and mechanisms, not accessible with simpler methods. Here we define the transcriptional changes in epidermal equivalents caused by the treatment with nickel, a known human allergen and carcinogen. We find that nickel regulates a characteristic set of genes, and that among these are prominently featured those associated with cell proliferation, inhibition of apoptosis, extracellular matrix remodeling and those encoding secreted signaling proteins.

Analysis of the nickel-regulated genes suggests that the molecular mechanisms responsive to nickel include a downregulation of the NF κ B pathway, which is important in inflammation. Conversely, the AP1 transcription factor appears activated in response to nickel salts. The signaling cascade responsible for this activation is under investigation. AP1 plays a role in inducing cell proliferation. Accordingly, we find cell cycle and DNA replication genes to be induced by nickel; these are novel findings, not previously thought to be an effect of nickel.

Our results are consistent with the recent results of Schoeters et al. ([2006]) who, used in vitro-derived dendritic cells and also found induction of cell cycle proteins by nickel, but did not distinguish between the pro- and anti-apoptotic ones. Our results are at variance with the recent results by Cheng et al. ([2003]) who found no effect on the cell cycle machinery and the reverse effects on the apoptosis genes in immortalized peripheral lung epithelial cell. The differences may be due to the different target cells (e.g., primary vs. immortalized, simple vs. stratified epithelia), experimental design (3D-differentiating vs. monolayers, time course vs. single time point etc.) or other factors. These controversies are the target of our future studies.

Among the most important effects of nickel are the induction of proteases and suppression of protease inhibitors. Unexpectedly, we also find an inverse relationship between the nickel-regulated and the Interferon- γ regulated genes. We propose that the cumulative effects of these changes are to enhance the antigen presenting capabilities of the keratinocytes. This effect explains the strong and widespread role of nickel in allergic reactions in humans.

Perhaps as interesting are the transcriptional effects of nickel that we did not observe; most prominently, nickel did not regulate any of the epidermal differentiation genes. Neither induction

nor suppression of the cornification markers was seen, and the lipid synthesis and steroid metabolism genes were also not affected. On the other hand, equally conspicuous by their absence are the markers characteristic for the basal layer of the epidermis. Although the cell cycle genes are induced, we did not observe induction of integrins, BPAGs, basal cell specific junctional proteins etc. This suggests that the mechanisms regulating cell cycle per se can be disconnected from those defining the basal cell phenotype. Taken together, the data suggest that the treatment with nickel neither promotes nor inhibits epidermal differentiation. In this respect, the effects of nickel are unusual, when compared to the effects of most other extracellular agents we tested (Li et al., [2001]; Banno et al., [2003], [2004b],[c], [2005]; Finelt et al., [2005]; Molenda et al., [2006]; Radoja et al., [2006]; Gazel et al., [2006a],[b]).

References



- Banno T, Adachi M, Mukkamala L, Blumenberg M. 2003. Unique keratinocyte-specific effects of interferon-gamma that protect skin from viruses, identified using transcriptional profiling. *Antivir Ther* **8**: 541-554. [Links](#)
- Banno T, Gazel A, Blumenberg M. 2004a. Effects of tumor necrosis factor-alpha (TNF alpha) in epidermal keratinocytes revealed using global transcriptional profiling. *J Biol Chem* **279**: 32633-32642. [Links](#)
- Banno T, Gazel A, Blumenberg M. 2004b. The use of DNA microarrays in dermatology research. *Retinoids* **20**: 1-4. [Links](#)
- Banno T, Gazel A, Blumenberg M, Adachi M, Mukkamala L. 2004c. Effects of tumor necrosis factor- α (TNF α) in epidermal keratinocytes revealed using global transcriptional profiling. *J Biol Chem* **279**: 32633-32642. [Links](#)
- Banno T, Gazel A, Blumenberg M. 2005. Pathway-specific profiling identifies the NF- κ B-dependent tumor necrosis factor α -regulated genes in epidermal keratinocytes. *J Biol Chem* **280**: 18973-18980. [Links](#)
- Bernard FX, Barrault C, Deguercy A, De Wever B, Rosdy M. 2000. Development of a highly sensitive in vitro phototoxicity assay using the SkinEthic reconstructed human epidermis. *Cell Biol Toxicol* **16**: 391-400. [Links](#)
- Blaschko A. 1889. Die Berufsdermatosen der Arbeiter: ein Beitrag zur Gewerbehygiene. I. Galvaniseur-Ekzem. *Dtsch Med Wochenschr* **15**: 925-927. [Links](#)
- Blumenberg M. 2005. Skinomics. *J Invest Dermatol* **124**: viii-x. [Links](#)
- Bolstad BM, Irizarry RA, Astrand M, Speed TP. 2003. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* **19**: 185-193. [Links](#)
- Cahan P, Ahmad AM, Burke H, Fu S, Lai Y, Florea L, Dharker N, Kobrinski T, Kale P, McCaffrey TA. 2005. List of lists-annotated (LOLA): A database for annotation and comparison of published microarray gene lists. *Gene* **360**: 78-82. [Links](#)
- Cavani A, Nasorri F, Ottaviani C, Sebastiani S, De Pita O, Girolomoni G. 2003. Human CD25+ regulatory T cells maintain immune tolerance to nickel in healthy, nonallergic individuals. *J Immunol* **171**: 5760-5768. [Links](#)
- Chen F, Ding M, Castranova V, Shi X. 2001. Carcinogenic metals and NF- κ B activation. *Mol Cell Biochem* **222**: 159-171. [Links](#)
- Cheng RY, Zhao A, Alvord WG, Powell DA, Bare RM, Masuda A, Takahashi T, Anderson LM, Kasprzak KS. 2003. Gene expression dose-response changes in microarrays after exposure of human peripheral lung epithelial cells to nickel(II). *Toxicol Appl Pharmacol* **191**: 22-39. [Links](#)
- Costa M, Yan Y, Zhao D, Salnikow K. 2003. Molecular mechanisms of nickel carcinogenesis: Gene silencing by nickel delivery to the nucleus and gene activation/inactivation by nickel-induced cell signaling. *J Environ Monit* **5**: 222-223. [Links](#)

Costa M, Davidson TL, Chen H, Ke Q, Zhang P, Yan Y, Huang C, Kluz T. 2005. Nickel carcinogenesis: Epigenetics and hypoxia signaling. *Mutat Res* **592**: 79-88. [Links](#)

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. 2003. DAVID: Database for annotation, visualization, and integrated discovery. *Genome Biol* **4**: P3 [Epub 2003 April 2003]. [Links](#)

Ding J, Zhang X, Li J, Song L, Ouyang W, Zhang D, Xue C, Costa M, Melendez JA, Huang C. 2006. Nickel compounds render anti-apoptotic effect to human bronchial epithelial beas-2B cells by induction of cyclooxygenase-2 through an IKK β /p65-dependent and IKK α - and p50-independent pathway. *J Biol Chem* **281**: 39022-39032. [Links](#)

Durham TR, Snow ET. 2006. Metal ions carcinogenesis. *EXS* **96**: 97-130. [Links](#)

Finelt N, Gazel A, Gorelick S, Blumenberg M. 2005. Transcriptional responses of human epidermal keratinocytes to Oncostatin-M. *Cytokine* **31**: 305-313. [Links](#)

Gandarillas A, Watt FM. 1997. c-Myc promotes differentiation of human epidermal stem cells. *Genes Dev* **11**: 2869-2882. [Links](#)

Garner LA. 2004. Contact dermatitis to metals. *Dermatol Ther* **17**: 321-327. [Links](#)

Gazel A, Ramphal P, Rosdy M, De Wever B, Tornier C, Hosein N, Lee B, Tomic-Canic M, Blumenberg M. 2003. Transcriptional profiling of epidermal keratinocytes: Comparison of genes expressed in skin, cultured keratinocytes, and reconstituted epidermis, using large DNA microarrays. *J Invest Dermatol* **121**: 1459-1468. [Links](#)

Gazel A, Banno T, Walsh R, Blumenberg M. 2006a. Inhibition of JNK promotes differentiation of epidermal keratinocytes. *J Biol Chem* **281**: 20530-20541. [Links](#)

Gazel A, Rosdy M, Bertino B, Tornier C, Sahuc F, Blumenberg M. 2006b. A characteristic subset of psoriasis-associated genes is induced by oncostatin-M in reconstituted epidermis. *J Invest Dermatol* **17**: 17. [Links](#)

Gueniche A, Viac J, Lizard G, Charveron M, Schmitt D. 1994. Effect of nickel on the activation state of normal human keratinocytes through interleukin 1 and intercellular adhesion molecule 1 expression. *Br J Dermatol* **131**: 250-256. [Links](#)

Haider AS, Peters SB, Kaporis H, Cardinale I, Fei J, Ott J, Blumenberg M, Bowcock AM, Krueger JG, Carucci JA. 2006. Genomic analysis defines a cancer-specific gene expression signature for human squamous cell carcinoma and distinguishes malignant hyperproliferation from benign hyperplasia. *J Invest Dermatol* **126**: 869-881. [Links](#)

Hirota M, Moro O. 2006. MIP-1 β , a novel biomarker for in vitro sensitization test using human monocytic cell line. *Toxicol In Vitro* **20**: 736-742. [Links](#)

Ho Sui SJ, Mortimer JR, Arenillas DJ, Brumm J, Walsh CJ, Kennedy BP, Wasserman WW. 2005. oPOSSUM: Identification of over-represented transcription factor binding sites in co-expressed genes. *Nucleic Acids Res* **33**: 3154-3164 [Print 2005]. [Links](#)

Hogenesch JB, Chan WK, Jackiw VH, Brown RC, Gu YZ, Pray-Grant M, Perdew GH, Bradfield CA. 1997. Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. *J Biol Chem* **272**: 8581-8593. [Links](#)

Karaczyn AA, Golebiowski F, Kasprzak KS. 2006. Ni(II) affects ubiquitination of core histones H2B and H2A. *Exp Cell Res* **312**: 3252-3259. [Links](#)

Kasprzak KS, Bal W, Karaczyn AA. 2003a. The role of chromatin damage in nickel-induced carcinogenesis. A review of recent developments. *J Environ Monit* **5**: 183-187. [Links](#)

Kasprzak KS, Sunderman FW Jr, Salnikow K. 2003b. Nickel carcinogenesis. *Mutat Res* **533**: 67-97. [Links](#)

Kowara R, Karaczyn A, Cheng RY, Salnikow K, Kasprzak KS. 2005. Microarray analysis of altered gene expression in murine fibroblasts transformed by nickel(II) to nickel(II)-resistant malignant phenotype. *Toxicol Appl Pharmacol* **205**: 1-10. [Links](#)

- Krob HA, Fleischer AB Jr, D'Agostino R Jr, Haverstock CL, Feldman S. 2004. Prevalence and relevance of contact dermatitis allergens: A meta-analysis of 15 years of published T.R.U.E. test data. *J Am Acad Dermatol* **51**: 349-353. [Links](#)
- Li D, Turi TG, Schuck A, Freedberg IM, Khitrov G, Blumenberg M. 2001. Rays and arrays: The transcriptional program in the response of human epidermal keratinocytes to UVB illumination. *FASEB J* **15**: 2533-2535. [Links](#)
- Lisby S, Muller KM, Jongeneel CV, Saurat JH, Hauser C. 1995. Nickel and skin irritants up-regulate tumor necrosis factor-alpha mRNA in keratinocytes by different but potentially synergistic mechanisms. *Int Immunol* **7**: 343-352. [Links](#)
- Marchese C, Visco V, Aimati L, Cardinali G, Kovacs D, Buttari B, Bellocci M, Torrisi MR, Picardo M. 2003. Nickel-induced keratinocyte proliferation and up-modulation of the keratinocyte growth factor receptor expression. *Exp Dermatol* **12**: 497-505. [Links](#)
- Maxwell P, Salnikow K. 2004. HIF-1: An oxygen and metal responsive transcription factor. *Cancer Biol Ther* **3**: 29-35. [Links](#)
- Minang JT, Arestrom I, Zuber B, Jonsson G, Troye-Blomberg M, Ahlborg N. 2006. Nickel-induced IL-10 down-regulates Th1- but not Th2-type cytokine responses to the contact allergen nickel. *Clin Exp Immunol* **143**: 494-502. [Links](#)
- Molenda M, Mukkamala L, Blumenberg M. 2006. Interleukin IL-12 blocks a specific subset of the transcriptional profile responsive to UVB in epidermal keratinocytes. *Mol Immunol* **43**: 1933-1940. [Links](#)
- Nestle FO, Speidel H, Speidel MO. 2002. Metallurgy: High nickel release from 1- and 2-euro coins. *Nature* **419**: 132. [Links](#)
- Newman JC, Weiner AM. 2005. L2L: A simple tool for discovering the hidden significance in microarray expression data. *Genome Biol* **6**: R81. [Links](#)
- Oxholm A, Oxholm P, Avnstorp C, Bendtzen K. 1991. Keratinocyte-expression of interleukin-6 but not of tumour necrosis factor-alpha is increased in the allergic and the irritant patch test reaction. *Acta Derm Venereol* **71**: 93-98. [Links](#)
- Pratt MD, Belsito DV, DeLeo VA, Fowler JF Jr, Fransway AF, Maibach HI, Marks JG, Mathias CG, Rietschel RL, Sasseville D, Sherertz EF, Storrs FJ, Taylor JS, Zug K. 2004. North American Contact Dermatitis Group patch-test results, 2001-2002 study period. *Dermatitis* **15**: 176-183. [Links](#)
- Radoja N, Gazel A, Banno T, Yano S, Blumenberg M. 2006. Transcriptional profiling of epidermal differentiation. *Physiol Genomics* **5**: 5. [Links](#)
- Reid TM, Feig DI, Loeb LA. 1994. Mutagenesis by metal-induced oxygen radicals. *Environ Health Perspect* **102**: 57-61. [Links](#)
- Rosdy M, Clauss LC. 1990. Terminal epidermal differentiation of human keratinocytes grown in chemically defined medium on inert filter substrates at the air-liquid interface. *J Invest Dermatol* **95**: 409-414. [Links](#)
- Rosdy MBB, Butet V, Gibbs S, Poncet M, Darmon M. 1997. Retinoic acid inhibits epidermal differentiation when applied topically on the stratum corneum of epidermis formed in vitro by human keratinocytes grown on defined medium. *In Vitro Mol Toxicol* **11**: 39-47. [Links](#)
- Salnikow K, Kasprzak KS. 2005. Ascorbate depletion: A critical step in nickel carcinogenesis? *Environ Health Perspect* **113**: 577-584. [Links](#)
- Salnikow K, Davidson T, Zhang Q, Chen LC, Su W, Costa M. 2003. The involvement of hypoxia-inducible transcription factor-1-dependent pathway in nickel carcinogenesis. *Cancer Res* **63**: 3524-3530. [Links](#)
- Schoeters E, Nuijten JM, Van Den Heuvel RL, Nelissen I, Witters H, Schoeters GE, Van Tendeloo VF, Berneman ZN, Verheyen GR. 2006. Gene expression signatures in CD34+-progenitor-derived dendritic cells exposed to the chemical contact allergen nickel sulfate. *Toxicol Appl Pharmacol*

216: 131-149. [Links](#)

Schwarz G, Boehncke WH, Braun M, Schroter CJ, Burster T, Flad T, Dressel D, Weber E, Schmid H, Kalbacher H. 2002. Cathepsin S activity is detectable in human keratinocytes and is selectively upregulated upon stimulation with interferon-gamma. *J Invest Dermatol* **119**: 44-49. [Links](#)

Sebastiani S, Albanesi C, Nasorri F, Girolomoni G, Cavani A. 2002. Nickel-specific CD4(+) and CD8(+) T cells display distinct migratory responses to chemokines produced during allergic contact dermatitis. *J Invest Dermatol* **118**: 1052-1058. [Links](#)

Serveau-Avesque C, Martino MF, Herve-Grepinet V, Hazouard E, Gauthier F, Diot E, Lalmanach G. 2006. Active cathepsins B, H, K, L, and S in human inflammatory bronchoalveolar lavage fluids. *Biol Cell* **98**: 15-22. [Links](#)

Shimono A, Okuda T, Kondoh H. 1999. N-myc-dependent repression of *ndr1*, a gene identified by direct subtraction of whole mouse embryo cDNAs between wild type and N-myc mutant. *Mech Dev* **83**: 39-52. [Links](#)

Stojadinovic O, Lee B, Vouthounis C, Vukelic S, Pastar I, Blumenberg M, Brem H, Tomic-Canic M. 2006. Novel genomic effects of glucocorticoids in epidermal keratinocytes: Inhibition of apoptosis, IFNgamma pathway and wound healing along with promotion of terminal differentiation. *J Biol Chem* **281**: 9. [Links](#)

Stojadinovic O, Lee B, Vouthounis C, Vukelic S, Pastar I, Blumenberg M, Brem H, Tomic-Canic M. 2007. Novel genomic effects of glucocorticoids in epidermal keratinocytes: Inhibition of apoptosis, interferon-gamma pathway, and wound healing along with promotion of terminal differentiation. *J Biol Chem* **282**: 4021-4034. [Links](#)

Tornier C, Rosdy M, Maibach HI. 2006. In vitro skin irritation testing on reconstituted human epidermis: Reproducibility for 50 chemicals tested with two protocols. *Toxicol In Vitro* **20**: 401-416. [Links](#)

Van Der Sluijs KF, Van Elden LJ, Arens R, Nijhuis M, Schuurman R, Florquin S, Kwakkel J, Akira S, Jansen HM, Lutter R, Van Der Polls T. 2005. Enhanced viral clearance in interleukin-18 gene-deficient mice after pulmonary infection with influenza A virus. *Immunology* **114**: 112-120. [Links](#)

Waikel RL, Wang XJ, Roop DR. 1999. Targeted expression of c-Myc in the epidermis alters normal proliferation, differentiation and UV-B induced apoptosis. *Oncogene* **18**: 4870-4878. [Links](#)

Wakem P, Burns RP Jr, Ramirez F, Zlotnick D, Ferbel B, Haidaris CG, Gaspari AA. 2000. Allergens and irritants transcriptionally upregulate CD80 gene expression in human keratinocytes. *J Invest Dermatol* **114**: 1085-1092. [Links](#)

Wang GL, Semenza GL. 1995. Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* **270**: 1230-1237. [Links](#)

Wiendl H, Lautwein A, Mitsdorffer M, Krause S, Erfurth S, Wienhold W, Morgalla M, Weber E, Overkleeft HS, Lochmuller H, Melms A, Tolosa E, Driessen C. 2003. Antigen processing and presentation in human muscle: Cathepsin S is critical for MHC class II expression and upregulated in inflammatory myopathies. *J Neuroimmunol* **138**: 132-143. [Links](#)

Zhou D, Salnikow K, Costa M. 1998. Cap43, a novel gene specifically induced by Ni²⁺ compounds. *Cancer Res* **58**: 2182-2189. [Links](#)

Zoroddu MA, Schinocca L, Kowalik-Jankowska T, Kozlowski H, Salnikow K, Costa M. 2002. Molecular mechanisms in nickel carcinogenesis: Modeling Ni(II) binding site in histone H4. *Environ Health Perspect* **110**: 719-723. [Links](#)