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Nuclear factor erythroid 2 – related factor 2 and its relationship with cellular response in nickel exposure: a systems biology analysis

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From 2nd Latin American Congress of Clinical and Laboratorial Toxicology
Porto Alegre, Brazil. 3-6 June 2018

Abstract

Background: Nickel and nickel-containing compounds (NCC) are known human carcinogens. However, the precise molecular mechanisms of nickel-induced malignant transformation remain unknown. Proposed mechanisms suggest that nickel and NCC may participate in the dual activation/inactivation of enzymatic pathways involved in cell defenses against oxidative damage, where Nuclear factor-erythroid 2 related factor 2 (Nrf2) plays a central role.

Methods: For assessing the potential role of proteins involved in the Nrf2-mediated response to nickel and NCC exposure, we designed an interactome network using the STITCH search engine version 5.0 and the STRING software 10.0. The major NCC-protein interactome (NCPI) generated was analyzed using the MCODE plugin, version 1.5.1 for the detection of interaction modules or subnetworks. Main centralities of the NCPI were determined with the CentiScape 2.2 plugin of Cytoscape 3.4.0 and main biological processes associated with each cluster were assessed using the BiNGO plugin of Cytoscape 3.4.0.

Results: Water-soluble NiSO₄ and insoluble Ni₃S₂ were the most connected to proteins involved in the NCPI network. Nrf2 was detected as one of the most relevant proteins in the network, participating in several multifunctional protein complexes in clusters 1, 2, 3 and 5. Ontological analysis of cluster 3 revealed several processes related to unfolded protein response (UPR) and response to endoplasmic reticulum (ER) stress.

Conclusions: Cellular response to NCC exposure was very comparable, particularly concerning oxidative stress response, inflammation, cell cycle/proliferation, and apoptosis. In this cellular response, Nrf2 was highly centralized and participated in several multifunctional protein complexes, including several related to ER-stress. These results add evidence on the possible Ni²⁺ induced – ER stress mainly associated with insoluble NCC. In this scenario, we also show how protein degradation mediated by ubiquitination seems to play key roles in cellular responses to Ni.

Keywords: Nickel, Nrf2, ER-stress, UPR, NiSO₄, Ni₃S₂

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Backgrounds

Nickel-containing compounds (NCC) are widely used in numerous industrial process while metallic nickel is a known human carcinogen [1]. However, the precise molecular mechanisms of nickel-induced malignant transformation remain unclear [2]. Toxicological profiles of NCC depend on their soluble and insoluble characteristics, which in turn affect its cellular uptake, and therefore the fate of dissolved nickel ion Ni^{2+} (carcinogen) [3]. Insoluble NCCs (i.e., Ni_3S_2 , $\text{Ni}(\text{CO})_4$) are taken up inside the cell via phagocytosis, were slow endosome solubilization leads to Ni^{2+} dissociation and aggregation near the nucleus, directly interacting with DNA [4].

In contrast, soluble compounds (i.e., NiSO_4 , NiCl_2) are incorporated via calcium/magnesium-dependent membrane channels, or the proton-coupled divalent cation transporter (DMT-1) and therefore affecting cytoplasmic proteins, which eventually causes increase cytotoxicity [5]. Cellular events following nickel and NCC exposure can be categorized as disrupted cell proliferation, metabolic modifications, oxidative stress, inflammation and cell death [6]. NCC can affect cellular iron uptake activating hypoxia signaling pathways (directly related nickel-induced carcinogenesis) and induce chemokine and cytokine gene expression (nickel-driven correlated pathway to immune response) [7, 8]. Several studies have concluded that production of reactive oxygen species (ROS) may also contribute to nickel-induced carcinogenesis [7], and recent studies have shown a link between environmental exposure to nickel compounds and increased oxidative DNA damage [9]. Proposed mechanisms suggest that nickel and nickel compounds may participate in the dual activation/inactivation of enzymatic pathways involved in cell defenses against oxidative damage, where the Nuclear factor-erythroid 2 related factor 2 (Nrf2) plays a central role [10]. Other reports indicate that Ni^{2+} at sublethal concentrations of 10–50 μM resembling in vivo tissue deposition can activate the Nrf2 signaling pathway in human monocytic cells by increasing whole-cell Nrf2 levels and nuclear translocation [11]. Nrf2 is a transcription factor that upregulates antioxidant response elements (AREs)-mediated expression of antioxidant enzyme and cytoprotective proteins, which protects the cells against oxidative injury in tissues and ROS [12]. Nrf2 has been traditionally considered as a tumor suppressor; however, several recent studies demonstrate that high Nrf2 overexpression may protect cancer cells against oxidative stress and chemotherapeutic agents [13]. Under oxidative stress conditions, Nrf2 is translocated to the nucleus, where it binds to AREs in the promoter regions of genes encoding antioxidant proteins and detoxification enzymes to initiate their transcriptions [14]. Additionally, recent evidence supports that

nickel-induced Nrf2 expression inhibits apoptosis and promotes autophagy, which may explain nickel tumorigenesis in vitro [15].

The use of hierarchical clustering analysis alongside with knowledge-based pathway information provides a comprehensive insight into the molecular mechanisms underlying metal-induced toxicity [16]. Thus, the present study aimed to evaluate the interaction between NCC, and proteins associated with the transcription factor Nrf2 using a systems biology approach, to elucidate how nickel and NCC exposure can affect the antioxidant response of the cell. The understanding of the complexity of the Nrf2 interactome could improve the identification of its role in the nickel-induced appearance of diseases such as cancer.

Methods

Construction of the chemo-biology network between nickel compounds and Nrf2-mediated response proteins

For assessing the potential role of proteins involved in the Nrf2-mediated response to nickel and NCC exposure, we designed an interactome network using the STITCH search engine version 5.0 and the STRING software 10.0 [17, 18]. While STITCH allows visualization of the physical interactions between the chemical elements and the proteins, the STRING software generates the protein-protein interactions [19].

Main NCC with potential toxicological characteristics detected in the workplace of the nickel-mining areas or nickel-using industries and its surrounding environments, [20–22] were used for network prospecting in STITCH. Compounds that were not present in the STITCH database (or those that did not show any protein binding) were excluded from the analysis. The parameters used to prospect the networks for *Homo sapiens* in STITCH software were: no more than 50 interactions; high confidence score (0.700); and network depth equal to 2; all active prediction methods enabled except text mining. Chemical names, CAS, synonyms and molecular formulae for the resulted NCC are presented in Table 1.

Protein interactions for Nrf2 were predicted in STRING 10.0. The parameters used to prospect the networks for *Homo sapiens* in STRING software were: no more than 50 interactions; high confidence score (0.700); and network depth equal to 2; all active prediction methods enabled except text mining. To identify additional interactors for Nrf2, we downloaded additional Nrf2 interactions deposited in Bio-GRID [23]. Each network generated by STITCH and STRING was combined in a single major NCC-protein interactome (NCPI) using the advanced network merge function of the Cytoscape 3.6.1 software [24].

Table 1 Chemical names and CAS names, synonyms, solubility and molecular formulae of selected nickel compounds

Chemical name	CAS Reg. No ^a	Synonyms	Formula	WSTC ^b (mg/L)
Nickel subsulfide	12035-72-2	Nickel sesquisulfide; tri-nickel disulfide; nickel subsulfide (Ni ₃ S ₂); nickel sulfide (Ni ₃ S ₂)	Ni ₃ S ₂	517 (37 °C) (Water-insoluble)
Nickel chloride	7718-54-9	Nickel (II) chloride; nickel (2+) chloride; nickel chloride (NiCl ₂); nickel dichloride; nickel dichloride (NiCl ₂); nickelous chloride	NiCl ₂	2,5E6 (20 °C) (Water-soluble)
Nickel sulfate	7786-81-4	Nickel monosulfate; nickelous sulfate; nickel sulfate (1:1); nickel (II) sulfate; nickel (2+) sulfate; nickel (2+) sulfate (1:1); nickel sulfate (NiSO ₄); sulfuric acid, nickel (2+) salt (1:1)	NiSO ₄	2,9E5 (20 °C) (Water-soluble)
Nickel carbonyl	13463-39-3	Nickel carbonyl (Ni(CO) ₄); nickel tetracarbonyl; tetracarbonylnickel; tetracarbonylnickel (0)	Ni (CO) ₄	20 (20 °C) (Water-insoluble)

^a [21]^b Water Solubility Temperature Corrected

Modular analysis of the interactome network

The major NCPI generated was analyzed using the MCODE (Molecular Complex Detection) plugin, version 1.5.1 for the detection of interaction modules or subnetworks (i.e., intensively connected parts of the network) [25]. Interaction modules may represent molecular complexes [26]. As previously described [27], t loops included; cutoff degree 2; deletion of single connected nodes from cluster (haircut option enabled); expansion of cluster by one neighbor shell allowed (fluff option enabled); node density cutoff 0.1; node score cutoff 0.2; kcore 2; and maximum depth of network 100. Compounds that were not present in the STITCH database (or those that did not show any protein binding) were excluded from the analysis.

Centralities analysis

To identify which nodes have a central position within the network, we performed the calculation of the main centralities of the network (degree, closeness and betweenness) with the CentiScape 2.2 plugin of Cytoscape 3.0 [28]. Betweenness indicates the number of the shortest paths that go through each node. Nodes with high betweenness scores are responsible for controlling the flow of information through the network topology; thus, more information will pass through that node [19]. Nodes with a high betweenness score are called bottleneck nodes (BN) and can represent essential proteins in signaling pathways [29]. On the other hand, node degree is a measure that indicates the number of connections associated with a specific node, which in turns, is also equal to the number of neighbors of the node [30]. Nodes with a relatively higher degree are termed hubs (H) [28] and have critical regulatory functions in the cell [19]. Consequently, a node with hub-bottleneck (H-BN) characteristics is considered a key regulator of a particular biological process and essential for the flow of information through the network [31].

Gene ontology (GO) analysis

In addition to the MCODE analysis, we analyzed the biological processes [32] associated with each cluster

using the Biological Network Gene Ontology (BiNGO) plugin of Cytoscape 3.4.0 [33]. The degree of functional enrichment for a given group and category was assessed quantitatively (*p*-value) using a hypergeometric distribution. The correction of the multiple tests was also evaluated by applying the false discovery rate (FDR) algorithm [34], which was fully implemented in the BiNGO software at a significance level of *p* < 0.05.

Results

The major NCPI generated for potential role proteins involved in the Nrf2-mediated response to NCC exposure included 188 nodes and 915 edges (Fig. 1). Water-soluble NiSO₄ and insoluble Ni₃S₂ were observed as the most protein linked NCC involved in the NCPI network. NiSO₄ is connected to 32 proteins with 118 connections and Ni₃S₂ is connected to 6 proteins with 11 connections.

The use of MCODE established the presence of 5 protein modules with a coefficient of cohesion greater than or equal to 3.0. Our analyses showed that in the cellular response to nickel exposure, Nrf2 is highly central and participates of several multifunctional protein complexes, as demonstrated by its simultaneous presence in clusters 1, 2, 3 and 5 (Fig. 2). It should be noted that after the MCODE analysis other NCC used in the initial prospection such as NiCO₄ did not display any clusterization, while NiCl₂ was found clustered in module 6 below the cutoff score and were excluded from the analysis. Additionally, cluster 5 did not show association to any NCC; thus, ontological processes related to this cluster were used only to discuss its relationship with cluster 3.

The system chemo-biology analysis of clusters 1, 2 and 3 revealed several common annotations: (i) *regulation of transcription*, (ii) *response to stress*, (iii) *regulation of cell proliferation*, (iv) *response to chemical stimulus*, (v) *regulation of cell death* and (vi) *response to oxidative stress*. All biological process linked to each of these clusters and its respective proteins are presented in (Additional file 1). In cluster 1, Nrf2 appeared associated with NiSO₄, while in cluster 2 was connected to Ni₃S₂ and NiSO₄, and in cluster 3 only to Ni₃S₂.

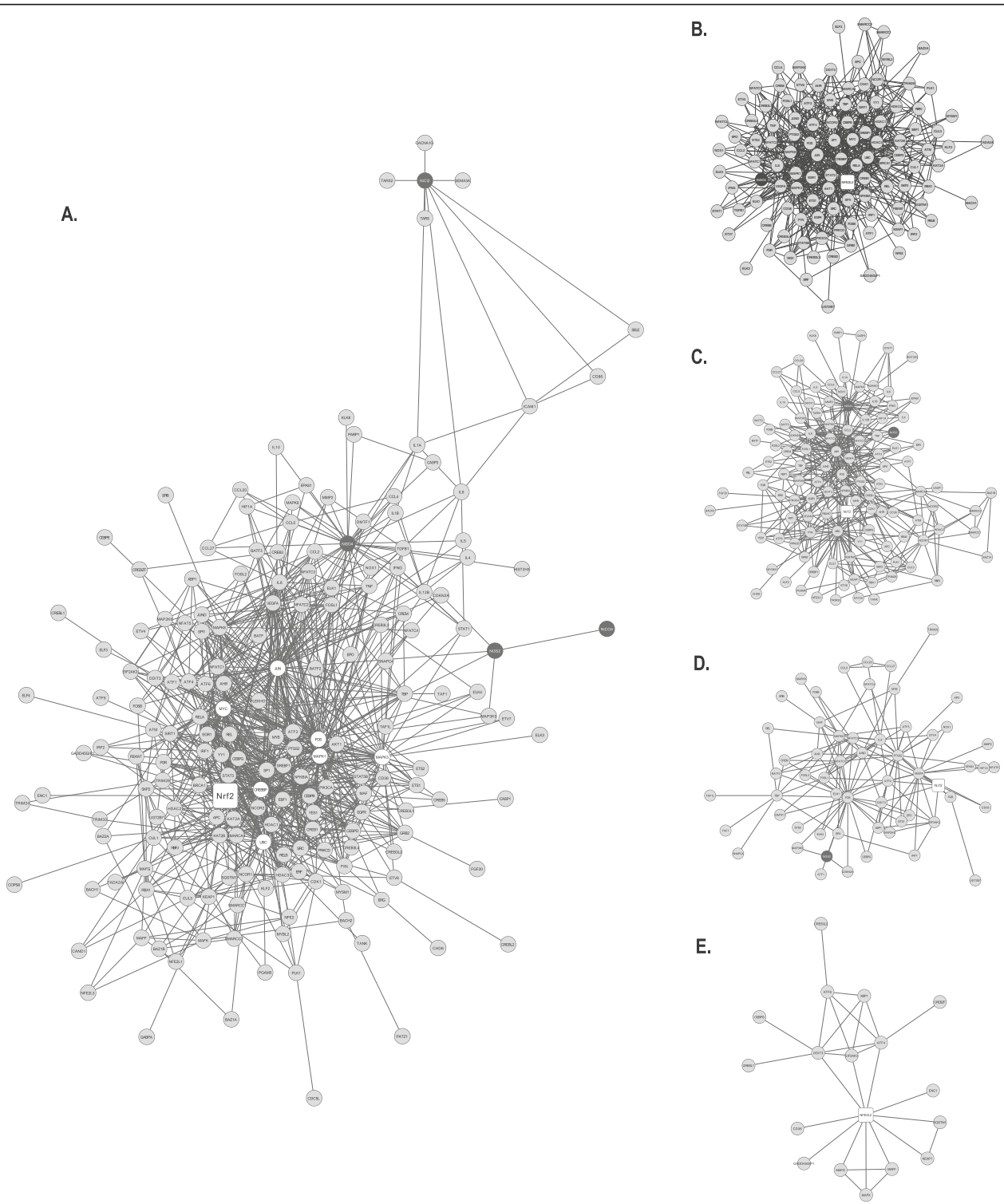
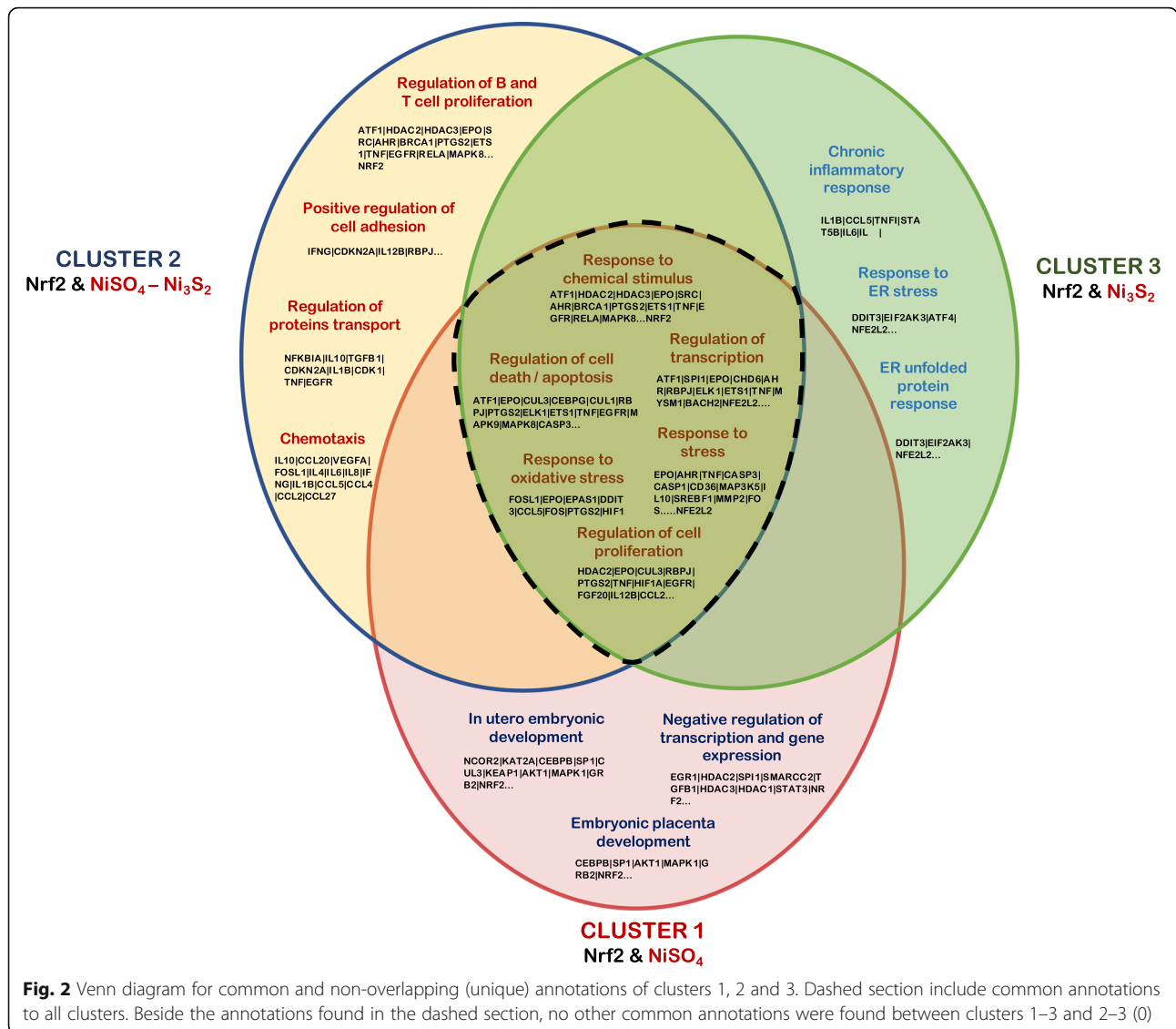


Fig. 1 The protein-protein interacting network of Ni₃S₂, NiCO₄, NiSO₄, and NiCl₂ with *Homo sapiens* proteins involved in the Nrf2-mediated response to nickel exposure. **a** The main network showing the NCC (black nodes) with 188 nodes and 915 edges (connections). HBs are represented by white nodes. Nrf2 appears as a round rectangle in white. **b** Cluster 1 composed of 114 nodes; 732 edges and associated to NiSO₄. **c** Cluster 2 composed of 113 nodes; 387 edges and associated to NiSO₄ and Ni₃S₂. **d** Cluster 3 composed of 55 nodes; 129 edges and associated to Ni₃S₂. **e** Cluster 5 composed of 18 nodes; 29 edges and no association with any NCC



Beside the common annotations described for all the clusters, we were interested in identifying mechanisms of toxicity that were unique to each NCC. Thus, a more detailed analysis of the GO terms in each cluster looking for non – overlapping (unique) annotations, revealed that in cluster 1, NiSO₄ differentially clustered in association with proteins involved with the negative regulation of transcription and gene expression, and interestingly, to proteins related to the development of the fetus and placenta (Fig.2). In this cluster, first Nrf2 neighboring proteins were also associated with the regulation of transcription and translation and response to stress (Data not shown).

In cluster 3, Ni₃S₂ were observed to be connected distinctly with proteins associated with the chronic inflammatory response and the response to ER stress, including the unfolded protein response. In cluster 2,

the presence of both NCC seems to cause a distinct clusterization around proteins associated with the immune and inflammatory response (Fig. 2). Additional biological process linked to cluster 3 and its respective proteins are presented in (Additional file 1: Table S3). In this particular cluster, Nrf2 appears as a connection protein between nodes involved in stress response and detoxification pathways (MAFG, MAFK, MAFF) and activation of signaling proteins involved in unfolded protein and DNA damage response (XBP1, ATF4, DDIT3, EIF2AK3).

To identify the significant nodes within the NCPI network, we calculated node degree, closeness, and betweenness centralities. From these analyses, two graphs were obtained containing the proteins that showed the highest centrality values (Fig.3 and Fig.4). From these analyses, we identified 10 major nodes with the highest

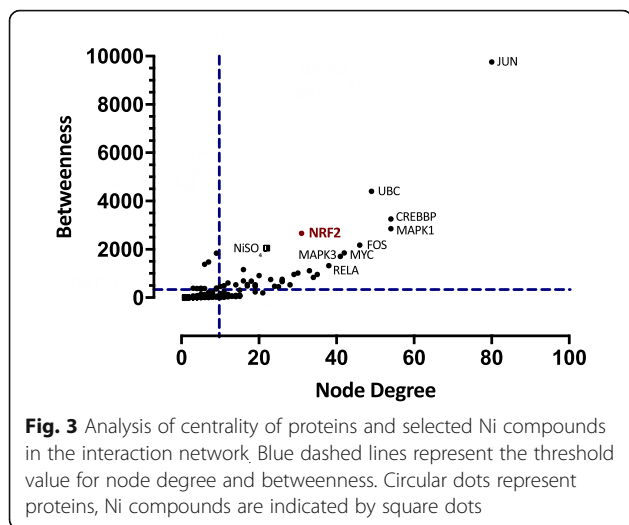


Fig. 3 Analysis of centrality of proteins and selected Ni compounds in the interaction network. Blue dashed lines represent the threshold value for node degree and betweenness. Circular dots represent proteins, Ni compounds are indicated by square dots

centrality values and H-BN characteristics: JUN, UBC, CREBBP, MAPK1, Nrf2, FOS, NiSO₄, MYC, MAPK3 and RELA (Fig. 3). Proteins described in clusters 1, 2, 3 and 5 and those with the highest centrality values were used in the design of a molecular model for potential role proteins involved in the Nrf2-mediated response to NCC exposure (Fig. 5).

Discussion

Considering that toxicological profiles of water soluble and insoluble NCC differ substantially, we decided to include both kinds of compounds in our analysis. However, only NiSO₄ and Ni₃S₂ were observed as the most connected to proteins involved in the NCPI network. NiSO₄ is a highly water-soluble nickel salt that causes a rapid release of Ni (II) ions after inhalation, while Ni₃S₂ is poorly soluble in water [3]. Soluble nickel particles can be dissolved in the lung and intestine tissues, and the nickel ions are quickly removed by ciliary transport.

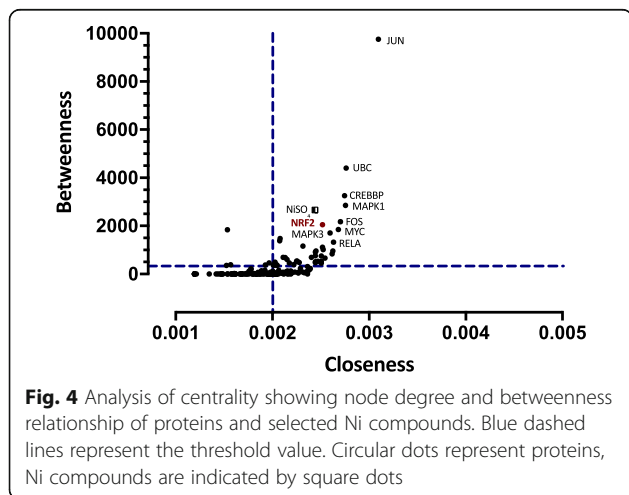


Fig. 4 Analysis of centrality showing node degree and betweenness relationship of proteins and selected Ni compounds. Blue dashed lines represent the threshold value. Circular dots represent proteins, Ni compounds are indicated by square dots

In contrast, less soluble nickel particles can enter epithelial cells by phagocytosis [35] where they are gradually dissolved, providing a constant source of nickel ions [36]. According to this “Ni ion bioavailability”-model, the carcinogenic potential of Ni depends on the availability of Ni ions in the cell nucleus [37]. Thus, NCC with the greatest potential to induce tumors would be those insoluble enough to be present in tissues as particles incorporated into epithelial cells via phagocytosis, and once inside the phagosomes capable of a slow, yet sustainable release over time of the Ni²⁺ ion [38, 39]. Phagocytic cells such as neutrophils and macrophages are important sources of endogenous oxygen radicals [40]. Thus, phagocytized nickel species, such as Ni₃S₂ and NiS produce more oxidative stress due to particle-induced irritation inside the cell [41, 42]; nevertheless, other NCC like NiSO₄ are also capable of inducing oxidative stress, especially in the presence of H₂O₂ [43]. Under these oxidative stress conditions, Nrf2 translocation to the nucleus activates ARE-mediated transcriptional response [14].

In our system chemo-biology analysis, Nrf2 appears as highly central protein, participating simultaneously of several multifunctional protein complexes. Remarkably, those cellular responses attributed to NiSO₄ and Ni₃S₂ were very similar among them particularly concerning oxidative stress response, and cell cycle/proliferation. However, a more detailed analysis of the GO terms in each cluster looking for non – overlapping (unique) annotations, revealed that in cluster 1, Nrf2 and NiSO₄ differentially clustered with proteins related to the development of the fetus and placenta. Regarding developmental toxicity, recent evidence supports that soluble Ni²⁺ can affect organogenesis in vivo [44–46], although nowadays no substantial epidemiological evidence has demonstrated a causal link between NiSO₄ and developmental effects [9]. As a contact allergen, NiSO₄ poorly correlated with Keap1/Nrf2 expression during dendritic cell activation in vitro [47]. However, nickel is capable of inducing lipid peroxidative damage in human placenta membrane, probably related to decreased placental viability, altered permeability and potentially subsequent embryotoxicity [48]. Previous studies demonstrated that nickel could decrease both intracellular glutathione levels and enzymatic activity of superoxide dismutase, glutathione peroxidase and catalase, which leads to ROS production and an increase in intracellular oxidative stress [49]. In this scenario, Nrf2 constitutes an essential regulator of placental development and growth. In a recent study, Kweider et al., [50] investigated the effects of Nrf2 on fetal-placental development in pregnant Nrf2 knockout and Nrf2 wild-type mice, finding that a deficiency in Nrf2 signaling can increase the level of oxidative stress and may negatively affect nutrient transfer

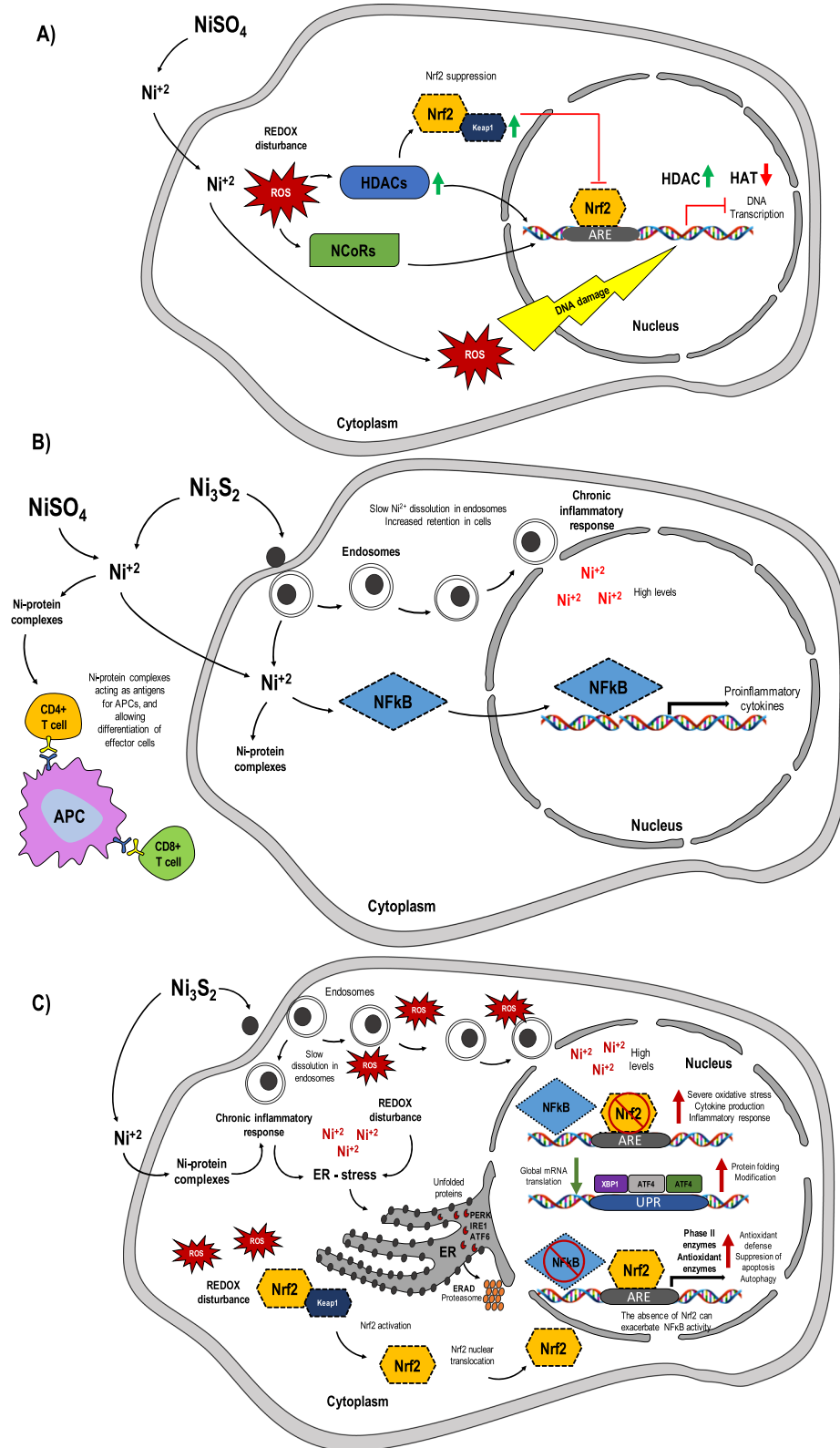


Fig. 5 (See legend on next page.)

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Fig. 5 Molecular models of the potential role of proteins involved in the Nrf2 – mediated response to NCC exposure. **a** NiSO₄ allows the release of Ni²⁺ ion which increases ROS production in the cytoplasm, inducing 1) hypomethylation and HAT inhibition through increased HDACs and NCoRs activity, which reduces Keap1/Nrf2 dissociation and posterior Nrf2/ARE transcription, and 2) inhibition of DNA repair process; **b**) Ni₃S₂ uptake is facilitated via phagocytosis, delaying Ni²⁺ dissolution inside the cytoplasm. However both Ni₃S₂ and NiSO₄ releases Ni²⁺ outside alveolar cells increasing production of Ni-protein complexes which acts as antigens for APC cells following differentiation to Ni²⁺-specific interferon γ (IFN-γ)-producing effector T cells, whereas inside the cell increase NF-κB transcription and proinflammatory response; **c**) Ni₃S₂ is capable of the release of high levels of the Ni²⁺ ion and ROS as a product of Ni₃S₂ mediated-phagocytosis. Retention of Ni₃S₂ and slow dissolution in endosomes are the primary cause of a possible chronic inflammation response. Ni²⁺ from Ni₃S₂ can induce ER stress through the activation of PERK, IRE1, and ATF6 pathways. During ER-stress conditions proteins that fail the ER quality control are transported back to the cytosol where they are rapidly destroyed by the ER-associated degradation (ERAD) pathway. Additionally, redox status generated by Ni²⁺ regulates the nuclear activity of NF-κB proteins by altering the binding of NF-κB proteins to DNA in part via the Nrf2-signaling pathway. The absence of Nrf2 can exacerbate NF-κB activity leading to increased cytokine production

capacity, affecting fetal growth demands. Other reports have suggested that nickel ions affect fetal development by altering maternal endocrine status leading to hyperglycemia [51]. As most of these results in human placenta have been reported for Ni and NiCl₂, our analysis would suggest similar effects for NiSO₄, which may suggest a potential new role of Nrf2-NiSO₄ correlation in other tissues during developmental stages.

In the same cluster 1, Nrf2 and NiSO₄ also differentially clustered in association with proteins involved with the negative regulation of transcription and gene expression. Several studies have also demonstrated that nickel species are also capable of negatively regulate transcription and gene expression through epigenetic mechanisms [52]. This aberrant histone modifications have been found to be associated with various human diseases, including cancer [53] and is considered as one of the possible mechanisms behind the potent carcinogenic effects of NCC [54]. Previous studies have shown that cellular exposure to NCC causes the intracellular accumulation of Ni ions and a loss of acetylation at all four core histones with global decreased DNA methylation [55, 56]. The evidence demonstrated that NCC exposure decreases histone acetylation by inhibiting histone acetyltransferase (HAT) activity but has no effects on histone deacetylases (HDAC) [57]. In this sense, within cluster 1 several class I HDAC (HDAC1, HDAC2, HDAC3) were identified, together with proteins with histone deacetylase activity like NCoR-1 and NCoR-2. Nickel species induce histone hypoacetylation through a multi-pathway, where cellular exposure to Ni²⁺ inhibits HAT activity via ROS production [57]. In this scenario, in vitro studies in cell lines of airway epithelial cells, demonstrated that increase HDAC activity correlated with lower levels of Nrf2 in parallel with elevated Nrf2 acetylation [58]. In line with this, other reports found that HDAC inhibition reduced the expression of Keap1, inducing Keap1/Nrf2 dissociation, Nrf2 nuclear translocation, and Nrf2 binding to ARE [59]. Thus, we can suppose that cellular exposure to NiSO₄ could increase HDAC activity and downregulation of the Nrf2 levels,

leading to oxidative damage, lipid peroxidation and DNA damage [60]. These modifications may at least partially account for NiSO₄ carcinogenic activities. Another possible explanation for NiSO₄ negative regulation of the transcription process in cluster 1, could include the direct effect of NiSO₄ on intracellular ROS generation and DNA damage repair inhibition [43]. In response to various types of DNA lesions, local inhibition of transcription may occur to prevent the production of aberrant transcripts and to avoid interference between transcription and repair machinery [61]. Additionally, considering that chromatin accessibility occurring after histone acetylation would allow repair proteins to gain access to the damaged lesion [62], the inhibition of HAT together with the increased activity of the HDAC may difficult the access of repair machinery to the damage inhibiting the repair process. In this regard, NCC and particularly NiSO₄ have been consistently associated with a decreased DNA repair capacity [43] that also can lead to a reduced transcription rate while the cell repairs the damaged DNA.

In cluster 2, Nrf2, NiSO₄, and Ni₃S₂ clustered in association with proteins involved with the immune and inflammatory response (TNF, NF-κB, IL4, IL6, IL8, and IFN) typically related with hypersensitivity to Ni-species. Both NiSO₄ and Ni₃S₂ are potent metallic allergens [53]. In NiSO₄ and other soluble NCC, Ni²⁺ can bond to soluble proteins on antigen-presenting cells (APC) [63]. APCs recognize this Ni²⁺-protein complex as an antigen, causing the activation of naïve T cells [64], which subsequently differentiate to Ni²⁺-specific interferon γ (IFN-γ)-producing CD4⁺ and CD8⁺ effector T cells [65], involved in Ni allergies. Ni²⁺ also activates the expression of intercellular adhesion molecules on endothelial cells and keratinocytes, therefore amplifying the inflammatory response [66]. More recently, Ni²⁺ and particularly NiSO₄ has been shown to alter inflammatory cytokine secretion from monocytes [67] by modulating transcription factor signaling pathways such as the nuclear factor kappa B (NF-κB), that regulate cytokine secretion [68, 69]. However, Ni²⁺ does not alter the activation or

nuclear translocation efficiency of NF- κ B [70]; emerging evidence suggest that redox status generated by Ni²⁺ regulates the nuclear activity of NF- κ B proteins [71] by altering the binding of NF- κ B proteins to DNA [70]. These reports suggest that Ni²⁺ could exert its effects on cytokine secretion at least in part via the Nrf2-signaling pathway [11, 71]. Furthermore, although Nrf2 absence can exacerbate NF- κ B activity by increasing cytokine production [72, 73], NF- κ B can modulate Nrf2 transcription and activity, having both upregulation and down-regulation effects on the target gene expression [74].

In cluster 3, Ni₃S₂ were observed to be connected distinctly with proteins associated with the chronic inflammatory response. In contrast to soluble NCC, Ni₃S₂ could be retained in lung cells for a sufficient time to exacerbate the development of inflammation response [75] and activate various signaling pathways that enhance allergic reactions as the second signal [76]. A chronic inflammation process is in accordance with the water-insolubility properties of Ni₃S₂ discussed above, which explains why inhalation of this form of nickel might be more related with a chronic response than inhalation of soluble nickel compounds [77].

Ni₃S₂ were also related to endoplasmic reticulum (ER) stress response, including the unfolded protein response (UPR). Several of these processes related to the response to ER stress were also found in cluster 5, although without association with any NCC. ER is the site of intracellular calcium regulation, synthesis, and folding of secretory proteins, which is crucial for cellular homeostasis [78]. Many disturbances, including those of cellular redox regulation, cause accumulation of unfolded proteins in the ER, affecting a variety of cellular signaling processes, including redox homeostasis, energy production, inflammation, differentiation, and apoptosis [79]. In consequence, ER stress activates the UPR to reestablish normal ER function by enhancing ER protein folding, inhibiting global mRNA translation, and reducing the influx of new proteins into the ER, which disposes terminally misfolded proteins by ER-associated protein degradation (ERAD) and autophagy [78]. Excessive and prolonged ER stress triggers cell suicide, usually in the form of apoptosis.

Although recent reports have demonstrated that As, Cd, Ag, Mn, and Cu can induce ER stress [80–83], there is limited evidence on Ni and NCC induced – ER stress, the latter only confirmed for nickel acetate in vitro [84] and NiCl₂ in vivo [85]. Based on our results and considering that metal ions can cause protein misfolding and aggregation by *i*) inhibiting the refolding of chemically denatured proteins in vitro, *ii*) interfering with protein folding in vivo and *iii*) causing the aggregation of nascent proteins in living cells [86], we can hypothesize that Ni²⁺ can activate the response to ER stress by meanings

of any of these mechanisms. Mainly, Ni₃S₂ has been associated with a more efficient uptake and delivery of Ni²⁺ ion to the cell nucleus in vitro compared to soluble NCC like NiSO₄ [87]. Additionally, Ni₃S₂ can indirectly induce ROS via inflammation and phagocyte activation, whereas directly through H₂O₂ formation [88]. Accumulating evidence suggests that protein folding and generation of ROS as a byproduct of protein oxidation in the ER are closely linked events [89]. It has also become apparent that UPR activation mediated by oxidative stress is an adaptive mechanism to preserve cell function and survival. In fact, persistent oxidative stress and subsequent ER stress and Nrf2 activation, initiate apoptotic cascades and are now known to play predominant roles in the pathogenesis of multiple human diseases including cancer, diabetes, atherosclerosis, and neurodegenerative diseases [90, 91]. However, the particular capacity of Ni₃S₂ induced ER stress and UPR response is of particular interest and needs to be further evaluated by in vitro studies.

Finally, to identify the major nodes within the NCPI, we calculated node degree, closeness and betweenness centralities. From these analyses, two graphs were obtained containing the proteins that showed the highest centrality values (Fig.3 and Fig.4). Interestingly, the same nodes showed similar relevance in both graphics. Thus, the joint analysis allows us to establish proteins that make up the most relevant network (hub-bottlenecks - HB). We identified several JUN, UBC, CREBBP, MAPK1, Nrf2, FOS, MYC, RELA, MAPK3 and NiSO₄ as HB. JUN (c-Jun, JunB, and JunD) and FOS family members (c-Fos, FosB, Fra1 and Fra2) constitute a mixture of homo and heterodimers that composed the AP-1 transcription factor. The AP-1 transcription factor participates in the control of cellular responses to stimuli that regulate proliferation, differentiation, immune response, cell death and the response to genotoxic agents or stress, all related with NCC response according to our results [92]. Apart from dimerize between each other, JUN and FOS proteins also dimerize efficiently with other transcription factors such as members of the ATF/CREB [93]. In fact, AP-1 can be induced in response to certain metals including nickel [94]. Expression of JunB was significantly up-regulated in some nickel exposures [95]. In human airway epithelial cells (BEAS-2B), c-Jun and c-Fos mRNA levels were induced after the addition of nickel [96]. AP-1 is a redox-sensitive transcription factor, both ROS-dependent and ROS-independent pathways may play a role in activation of AP-1 in nickel-exposed cells [97, 98]. This ROS-mediated activation of AP-1 is modulated by the transcription activation of APE, the redox regulator of AP-1 and is mediated via activation of c-Jun/ATF-2 that binds to the CREB binding site within the human APE promoter [99]. CREB is a leucine

zipper-type transcription factor also found as HB in the NCPI, ubiquitously expressed in organs [100]. CREB has many functions in cells, including regulation of cell growth, differentiation and stress responses in various cell types, and recently has been strictly associated with the ER stress [101]. In line with this, another HB protein, ubiquitin C (UBC) has been shown to be involved in the regulation of various biological processes related to nickel-induced toxicity [102], and other biological processes such as the cell cycle, apoptosis, and inflammation, which are contributors to human cell toxicity and carcinogenicity [103]. The ubiquitin – proteasome pathway (UPP) catalyzes the selective degradation of short-lived proteins and the elimination of proteins with abnormal conformation [104], including those that accumulate in ER during ER-stress conditions; this process is known as ER-associated degradation (ERAD), proteins that fail the ER quality control are transported back to the cytosol where they are rapidly destroyed by the UPP [105]. MYC is a family of proto-oncogenes that code for transcription factors, known to induce both increase proliferation and apoptosis depending on cellular status [106]. Particularly, c-Myc functions as a transcription factor and induces proliferation, energy metabolism [107], and ribosome biogenesis [108]. This ribosome biogenesis has been associated with an enhanced protein synthesis, required for MYC oncogenic activity [109, 110] and more recently with UPR activation [106]. Thus, UPR would act as an enhancer of c-Myc-induced transformation, suggesting that UPR inhibition may be particularly effective against malignancies characterized by c-Myc overexpression [106]. RELA is one of the five different proteins of the NF- κ B factor previously discussed, also called p65 subunit. p65 plays a critical role in inducing target genes of NF- κ B; recent evidence has demonstrated the translocation of NF- κ B p65 following ER stress [111]. Additionally, this subunit has been shown to function as a negative regulator of Nrf2 activation either by depriving CREB binding protein from Nrf2 or by recruitment of histone deacetylase 3 (HDAC3), a corepressor to ARE [112]. In another study it was shown that p65 decreased NRF2 through the interaction of Keap1, inhibiting Nrf2-ARE pathway [74].

Conclusions

Our analysis based on system chemobiology tools suggest that during NCC exposure, Nrf2 would play a pivotal role during cellular transcriptional, regulatory and chemical responses, processes correlated with cell proliferation, death, and oxidative stress. Our results add some evidence to soluble NCC and its relationship with modification of the epigenetic response regarding ROS-mediated histone acetylation/deacetylation, which may be related to Nrf2 suppression, a process that may be

surpassing DNA repair mechanisms inside the cell. Compared with the response to water-soluble NCC exposure, Nrf2 seems to play a more significant role in response to insoluble NCC where phagocytized nickel species may produce and increase oxidative stress, chronic inflammation and ER- stress. These results may suggest a model where Ni exposure may cause persistent oxidative stress, subsequent ER stress, and Nrf2 activation, adding evidence on the possible Ni²⁺ induced – ER stress. Additionally, in this model, protein degradation mediated by ubiquitination seems to play key roles in cellular responses to Ni. Thus, our results would suggest that water-insolubility of NCC plays a major role in predominant cell damage regarding ER stress, and in general, a systemic effect of NCC based on solubility as revealed by cluster analysis.

Furthermore, our model provides new insights regarding how NCC obtained as by-products in many industrial processes may cause affectations regarding chronic response to several diseases.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s40360-019-0360-4>.

Additional file . clusters ontology analysis.

Abbreviations

ARE: antioxidant response elements; ER: endoplasmic reticulum; HAT: histone acetyltransferase; HDAC: histone deacetylase; NCC: nickel containing compounds; NCPI: NCC-protein interactome; NiSO₄: nickel sulfate; Ni₃S₂: nickel subsulfate; NiCl₂: nickel chloride; Ni (CO)₄: nickel carbonyl; Nrf2: Nuclear factor-erythroid 2 related factor 2; ROS: reactive oxygen species; UPR: unfolded protein response

Acknowledgements

The authors thanks to Universidad del Sinú – Elías Bechara Zainúm for providing the financial support for this research.

About this supplement

This article has been published as part of *BMC Pharmacology and Toxicology Volume 20 Supplement 1, 2019: Proceedings of Toxi-Latin 2018*. The full contents of the supplement are available online at <https://bmcpharmacolotoxicol.biomedcentral.com/articles/supplements/volume-20-supplement-1>.

Author's contribution

LEP proposed and conceived the analysis. LEP, JTA, LJV and PEP construct the networks, analyzed, and interpreted the data. LJV wrote the manuscript with support of DRC, SSA. Both CGP, KPS contributed to the final version of the manuscript. All authors discussed the results and contributed to the final manuscript.

Funding

Publication costs are funded by Universidad del Sinú – Elías Bechara Zainúm, Internal Research Grant - 2016.

Availability of data and materials

The databases constructed and used in this article are public and available in the supplemental material published.

Ethics approval and consent to participate

No

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Published: 19 December 2019

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