Roles of Nrf2 in Drug and Chemical Toxicity

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Abstract

The transcription factor Nrf2 regulates the basal and inducible expression of numerous cell defence genes, and thus protects mammalian cells against the deleterious effects of chemical and oxidative stress. Here, we provide a concise overview of the role of Nrf2 in protection against drug and chemical toxicity, with a focus on the numerous *in vivo*studies that have shown heightened sensitivity of Nrf2 null (knockout) transgenic mice to such insults. In light of the emerging role for Nrf2 in driving drug resistance in cancer, we also consider the potential benefits and risks of modulating Nrf2 activity to overcome chemoresistance in patients. Finally, we highlight recent efforts to monitor perturbation of the Nrf2 pathway *in vitro* as part of integrated risk assessment strategies designed to identify compounds that are hazardous to human health.

1. Introduction

Drug and chemical toxicity represent a significant human health hazard, with the former limiting the development and use of otherwise effective medicines [1]. Oxidative stress underpins the toxic effects of many chemical entities. However, mammalian cells are equipped with an antioxidant stress response pathway, regulated by the transcription factor nuclear factor erythroid 2 related factor 2 (Nrf2), to counteract the deleterious effects of oxidative stress. Here, we provide a brief overview of the Nrf2 pathway (other articles in this themed issue provide more detailed descriptions of molecular and regulatory features) and highlight its role in protection against drug and chemical toxicity.

2. The Nrf2 pathway

Nrf2 is a member of the cap 'n' collar (CNC) family of basic leucine zipper (bZIP) transcription factors that is evolutionarily conserved and expressed in essentially all tissues. Under normal conditions, Nrf2 is sequestered in the cytoplasm and targeted for ubiquitination and proteasomal degradation by its repressor Kelch-like ECH-associated protein 1 (Keap1). Under conditions of chemical or oxidative stress, reactive cysteine residues in Keap1 undergo redox modification, inhibiting Keap1-mediated degradation of Nrf2 and stabilising the transcription factor (Figure 1). The resulting accumulation of Nrf2 in the nucleus facilitates interaction with small Maf proteins and increases the transcription of cytoprotective genes that poses one or more antioxidant responsive elements (AREs) in their promoter regions [2]. More than 100 Nrf2-regulated genes have been identified to date, with many contributing to homeostatic processes including xenobiotic metabolism, glutathione synthesis/recycling, antioxidation and the provision of cellular fuels (Table 1). A role for Nrf2 in the regulation of these processes has largely been established through omics analyses of organs from transgenic $Nrf2^{-/-}$ mice, as we recently reported for the kidney [3]. By regulating the basal and inducible expression of this diverse set of genes, Nrf2 influences cell and organism sensitivity to a range of chemical and oxidative insults (Figure 1).

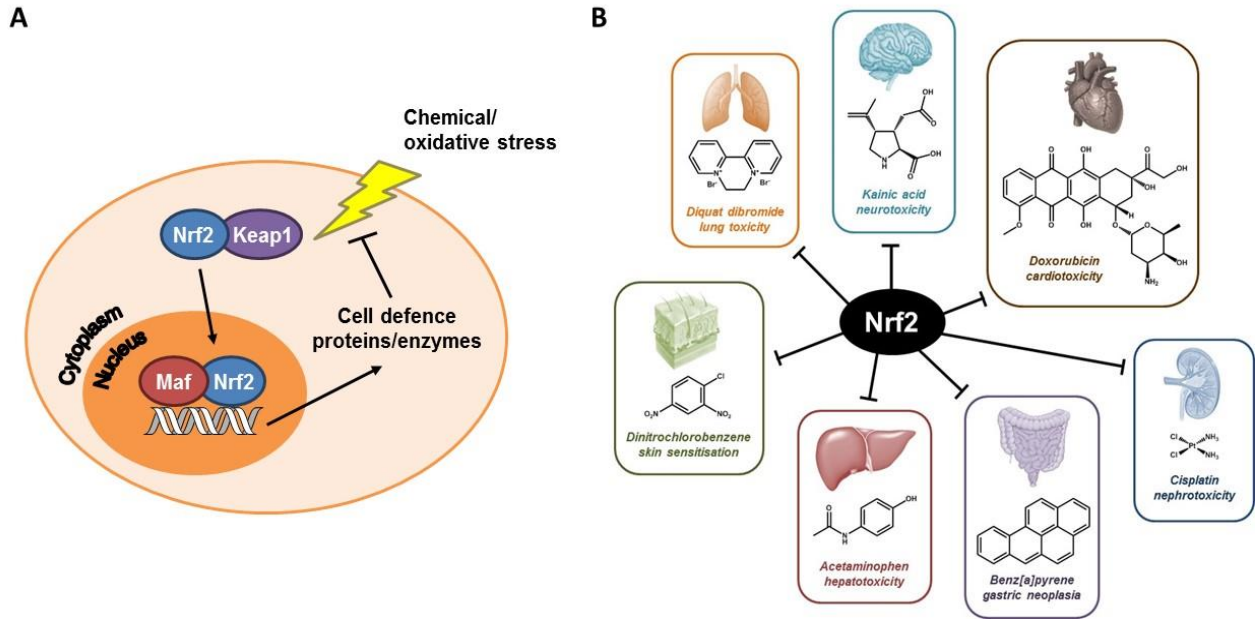

Figure 1 – The Nrf2 pathway and its influence on drug and chemical toxicity. *(A) In response to chemical/oxidative stress, Nrf2 evades Keap1-mediated repression and accumulates in the nucleus, where it dimerises with small Maf proteins and transactivates cytoprotective target genes that serve to detoxify the original insult and limit its deleterious effects. (B) Examples of drug and chemical toxicities against which Nrf2 protects, based on evidence from in vivo studies using Nrf2$^{-/-}$ mice (see Table 2 for other examples).*

Class	Example Gene	Example Gene Function
Phase I drug metabolism	NAD(P)H dehydrogenase quinone 1 (Nqo1)	Reduces reactive quinones to hydroquinones.
Phase II drug metabolism	UDP-glucuronyltransferases 1 family polypeptide A (Ugt1a1)	Catalyses conjugation of drugs and small molecules with glucuronic acid, increasing hydrophilicity.
Phase III drug metabolism	ATP-binding cassette sub-family C, member 1 (Abcc1)	Effluxes drugs and other small molecules from cells.
Glutathione metabolism	Glutamate cysteine ligase, catalytic subunit (Gclc)	Rate limiting enzyme in synthesis of glutathione, the major antioxidant in cells.
Antioxidant processes	Sulfiredoxin 1 (Srxn1)	Reduces oxidative modifications of cysteine residues in peroxiredoxins.
Heme metabolism	Heme oxygenase 1 (Hmox1)	Converts toxic free heme to biliverdin (subsequently converted to antioxidants bilirubin and carbon monoxide).
NADPH metabolism	6-Phosphogluconate dehydrogenase (Pgd)	Catalyses one step of pentose phosphate pathway.

Table 1 – Major classes and examples of cytoprotective genes regulated by Nrf2. *For details of these and other relevant genes, see [2].*

3. Nrf2 protects against drug and chemical toxicity – Positive aspects

The first *in vivo* demonstration of the protective effect of Nrf2 was in 1999, when Chan and Kan showed that $Nrf2^{-/-}$ mice developed acute respiratory distress syndrome following exposure to doses of butylated hydroxytoluene that were tolerated by wild type mice [4]. This was soon followed by reports that $Nrf2^{-/-}$ mice were highly sensitive to the model liver toxin acetaminophen 5 and 6, shown subsequently to be a result of an increase in its conversion to the reactive metabolite N-acetyl-p-benzoquinoneimine and a decrease in hepatic detoxification capacity [7]. To date, a large number of substances (Table 2) have been shown to provoke greater toxicity in major organs of $Nrf2^{-/-}$ mice, which lack Nrf2 in all tissues. Whilst many of these substances (e.g. carbon tetrachloride, ovalbumin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) are primarily experimental tools used to study organ injury, several investigations have focused on the role of Nrf2 in protection against environmental agents (e.g. diquat dibromide, cigarette smoke, benzo[a]pyrene) or therapeutic drugs (e.g. bleomycin, methamphetamine, cisplatin) with adverse effects that are relevant to man. The large body of evidence from studies using $Nrf2^{-/-}$ mice has been further supported by investigations involving the genetic or pharmacological upregulation of Nrf2 activity. For example, both hepatocyte-specific loss of the Keap1 gene [8] and pre-treatment with the triterpenoid Nrf2 inducer 2-cyano-3,12-dioxooleana-1,9-dien-28-oic imidazolide (CDDO-Im; [9]) affords protection against acetaminophen liver injury. Therefore, whilst some organs (e.g. liver and lung) have been studied more extensively than others, it is clear that Nrf2 exerts a general protective effect against drug and chemical toxicity in mice (Figure 1). Based on these observations and work in human cell systems, there is a tacit assumption that Nrf2 plays an equally important role in man, although there have been very few investigations in this context, and it will be important to determine the importance of, and potential value of targeting, Nrf2 in human drug and chemical toxicities.

Target Organ	Compound	Phenotype of Nrf2$^{-/-}$ Mice	Reference(s)
Liver	Acetaminophen	Increased hepatocellular necrosis	[5,6,9-12]
	2-Amino-3-methylimidazo[4,5-f]quinolone	Increased incidence and multiplicity of hepatocellular adenoma/carcinoma	[13]
	1-Bromopropane	Increased hepatocellular necrosis	[14]
	Cadmium chloride	Increased hepatic haemorrhage/necrosis	[11,15]
	Carbon tetrachloride	Delayed repair of hepatic necrosis	[11,16]
	Diquat dibromide	Increased hepatocellular necrosis	[17]
	Ferric ammonium citrate	Increased hepatic siderosis/necrosis	[18]
	Furosemide	Increased hepatocellular necrosis	[11,19]
	Lapatinib	Increased serum alanine/aspartate aminotransferase levels	[20]
	Lithocholic acid	Increased hepatocellular/ductal necrosis	[11,21]
	Pentachlorophenol	Increased hepatocellular adenoma and cholangio- fibrosis/carcinoma	[22,23]
	Phalloidin	Increased hepatic haemorrhage/necrosis	[11,24]
	Piperonyl butoxide	Increased hepatocellular adenoma	[22]
	Pyrazole	Increased hepatocellular necrosis	[25]
	Sodium arsenite	Increased hepatic necrosis	[26]
	2,3,7,8-Tetrachlorodibenzo-p-dioxin	Increased hepatic degeneration/ inflammation	[27]
Lung	Bleomycin	Increased pulmonary inflammation/ fibrosis	[28,29]
	Butylated hydroxytoluene	Increased pulmonary haemorrhage and alveolar destruction	[4]
	Carrageenan	Increased alveolar edema and neutrophil infiltration	[30,31]
	Cigarette smoke	Increased alveolar destruction	[32-34]
	Diesel exhaust particulate	Increased mucal cell hyperplasia	[35,36]
	Diquat dibromide	Increased alveolar edema/destruction	[17]
	Elastase	Increased pulmonary haemorrhage and alveolar destruction	[37]
	Lipopolysaccharide (LPS)	Increased pulmonary edema and neutrophil infiltration	[38,39]
	Multi-walled carbon nanotubes	Increased pulmonary inflammation and neutrophil infiltration	[40]
	Ovalbumin	Increased pulmonary inflammatory cell infiltration	[41]
	Titanium dioxide (TiO_2) nanoparticles	Increased pulmonary inflammatory cell infiltration	[42]
Brain	6-Hydroxydopamine	Increased striatal lesion volume	[43]
	Kainic acid	Increased seizure intensity and hippocampal neuron damage	[44]
	LPS	Increased hippocampal inflammation and microglial activation	[45]
	Malonic acid	Increased striatal lesion volume	[46]
	Methamphetamine	Increased gliosis and loss of striatal neurons	[47]
	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine	Increased gliosis and loss of striatal neurons	[48-51]
	3-Nitropropionic acid	Increase striatal lesion volume and decreased motor function	[46,52]
Kidney	Cisplatin	Increased proximal tubule degeneration/ necrosis	[53,54]
	Ferric nitrilotriacetate	Increased proximal tubule degeneration/ necrosis	[55,56]
	Pristine	Increased renal vascular permeability and glomerular autoantibody deposition	[57]
	Streptozotocin	Increased hyperglycemia and glomerulosclerosis	[58-60]
Skin	Cinnamaldehyde	Increased dermal edema and inflammatory cell infiltration	[61]
	7,12-Dimethylbenz(a)anthracene &	Increased incidence of skin tumours	[62]

	12-O-tetradecanoylphorbol-13-acetate		
	2,4-Dinitrochlorobenzene	Increased dermal edema and inflammatory cell infiltration	[61,63]
Bladder	N-nitrosobutyl(4-hydroxybutyl)amine	Increased incidence of invasive/non-invasive bladder carcinoma	[64,65]
	Sodium arsenite	Increased interstitial edema/congestion	[26]
GI tract	2-Amino-3-methylimidazo[4,5-f]quinolone	Increased incidence of forestomach squamous cell hyperplasia	[13]
	Benzo[a]pyrene	Increased incidence of forestomach neoplasia	[66,67]
	Dextran sulfate sodium	Increased loss of colonic crypts	[68,69]
Heart	Doxorubicin	Increased cardiac necrosis and loss of cardiac function	[70]

Table 2 – Drug and chemical toxicities, affecting major organs, which are exacerbated in Nrf2$^{-/-}$ mice. *Due to space constraints, single studies showing that Nrf2 protects against drug and chemical toxicities affecting the breast, ear, eye, foetus and ovary have been excluded. Given the focus of this article, studies demonstrating that Nrf2 protects against the adverse effects of mechanical injury (e.g. tissue trauma, ischemia-reperfusion), environmental insult (e.g. ultraviolet light, hyperoxia), inflammatory proteins or altered composition of diet have not been considered.*

4. Nrf2 protects against drug and chemical toxicity – Negative aspects

A increasing body of evidence indicates that Nrf2 pathway activity is elevated in a large proportion of cancers, due to somatic mutations in the Nrf2 or Keap1 genes that render the latter unable to efficiently repress Nrf2 [71]. By regulating the expression of genes that coordinate important metabolic processes, and enabling cells to survive in a highly oxidative environment via the upregulation of antioxidant genes, it appears that Nrf2 can promote cancer cell proliferation [71]. Another unwelcome consequence of the increase in Nrf2 activity in some tumours is their heightened levels of protective genes that serve to detoxify cytotoxic chemotherapeutics and antagonise their deleterious effects. A number of groups have provided proof-of-concept that inhibition of Nrf2 (most commonly via RNAi knockdown) can resensitize some cancer cells to a formerly efficacious anti-cancer agent [71]. These observations have generated interest in the potential for inhibiting Nrf2 as a means of overcoming drug resistance in cancer patients. Notwithstanding the fact that relatively few small molecule inhibitors of Nrf2 have been described, and that mechanisms of action are unclear in many cases [72], it is important to consider that many established chemotherapeutic drugs (e.g. cisplatin, bleomycin, doxorubicin; Table 2) are associated with off-target toxicities against which Nrf2 protects. Therefore, it is possible that systemic coadjuvant inhibition of Nrf2 could enhance cancer cell killing but also exacerbate adverse effects in other organs. To address this paradox, it will be important to explore therapeutic strategies through which Nrf2 can be modulated, ideally in a manner that specifically targets cancer cells, to provide a more favourable balance between efficacy and off-target toxicity. The identification of such strategies is vital to make the translation of this approach to patients more feasible.

5. Nrf2 directs an adaptive response to chemical and oxidative stress – Relevance to chemical risk assessment

The activity of Nrf2 is primarily regulated by its interaction with its redox-sensitive cytosolic repressor Keap1 [73]. We and others have provided direct evidence for the chemical modification of specific cysteine residues in Keap1 by electrophilic compounds that stimulate Nrf2 signalling [73]. It is thought that this ability to 'sense' reactive chemical entities and oxidative species underpins the rapid accumulation of Nrf2 protein and consequent increased expression of its target genes that are typically observed in cells exposed to chemical stressors, although other mechanisms have been proposed [73]. In an effort to exploit the responsiveness of the Nrf2 pathway to oxidative stress in order to improve human hazard prediction, in recent years several reporter cell lines have been engineered to stably express luciferase under the transcriptional control of one or more AREs cloned from the promoter region of an Nrf2 target gene. One

such platform, the KeratinoSens assay, has recently been approved by the Organisation for Economic Cooperation and Development (OECD) for use in an integrated testing strategy for the *in vitro* detection of skin sensitizers [74], an area that has seen rapid progress following the recent European Union ban on the use of animals for testing of cosmetic products. A different reporter cell line, known as CellSensor ARE-bla [75], is one of 30 being used in the Tox21 initiative to screen thousands of compounds in high-throughput with the aim of identifying structure-activity signatures that could be used to better predict toxicity in humans, and in turn reduce reliance on animal testing. By considering perturbation of the Nrf2 pathway alongside other important stress responses, and by multiplexing these assays with evaluation of cell viability to distinguish specific pathway activation from non-specific cytotoxic events, Tox21 promises to give a systems perspective to hazard identification that may shed light on important chemical and molecular distinctions between sets of compounds that commonly activate Nrf2 signalling yet are associated with beneficial (e.g. cytoprotective, chemopreventive) and deleterious (e.g. cytotoxic) effects in cells, animals and/or man. In many cases, these distinctions may be largely a function of concentration and time, but a deeper understanding will aid the identification of compounds with potential therapeutic applications and those that pose a risk of toxicity.

5. Future directions

The above represents a concise overview of our current understanding of the relationship between Nrf2 and chemical toxicity. Given the overwhelming evidence for a protective role of Nrf2 in mice, it will be necessary to establish whether a similar function is served in humans. In particular, it will be important to determine whether an individual's Nrf2 status predicts their susceptibility to a given drug or chemical toxicity. For example, single nucleotide polymorphisms in the human Nrf2 gene have been correlated with risk of various diseases [76]. It will be important to determine whether these or other polymorphisms render carriers more susceptible to the adverse effects of certain drugs. Based on the findings of these and other investigations, and dependent on the identification of more potent and specific Nrf2 modulators, it is possible that Nrf2 activity could be manipulated to improve the therapeutic index of a drug, by bolstering the endogenous buffer against its adverse effects or, in some cases, by improving its therapeutic efficacy. In the coming years, the challenge for pharmacologists and toxicologists is to harness an ever-increasing knowledge of pathway biology and a burgeoning clinical experience of targeting Nrf2 to aid the development of safe and effective new drugs and identify strategies for improving the use of existing medicines in patients.

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