Opposite effects of two zinc(II) dithiocarbamates on NF-kB pathway

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„Progress isn't based on knowledge, it is based on ideas."
Sir J. W. Black (Nobel Prize 1988)

Synthesis of complexes:

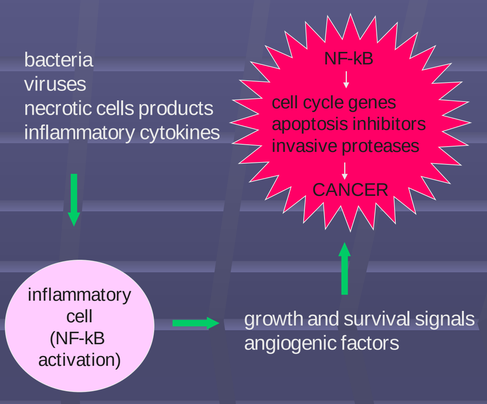
- aqueous solutions of $ZnCl_2$ and sodium diethyldithiocarbamate ($NaEt_2DTC$) or sodium dibenzyldithiocarbamate ($NaBz_2DTC$) were mixed at rate 1:2
- hard soluble powders immediately originated, which were carefully washed by distilled water until negative chloride test and dried by room temperature for several days to constant weight

X-ray analysis and mass spectroscopy:

- molecular structures of obtained compounds are in conformity with literature (Bonamico et al. *Acta Crystallogr* 1965; Decken et al. *Appl Organomet Chem* 2006) and are stable in aqueous milieu (according to APCI mass spectrometry)
- Zn(II) in both $[Zn(Et_2DTC)_2]$ and $[Zn(Bz_2DTC)_2]$ is chelated by 4 sulfurs with various coordination geometry: tetragonal planar (former) and tetrahedral (latter)

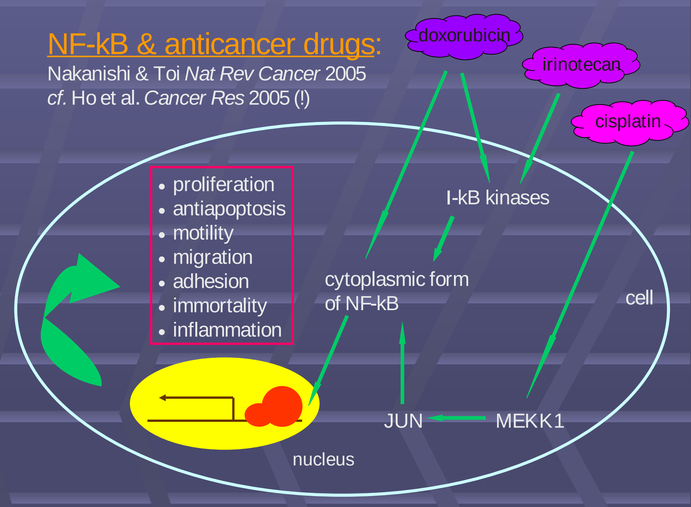
Nuclear factor-kappaB (NF-kB) & cancer:

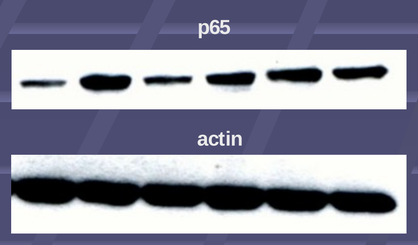
Karin *Nature* 2006

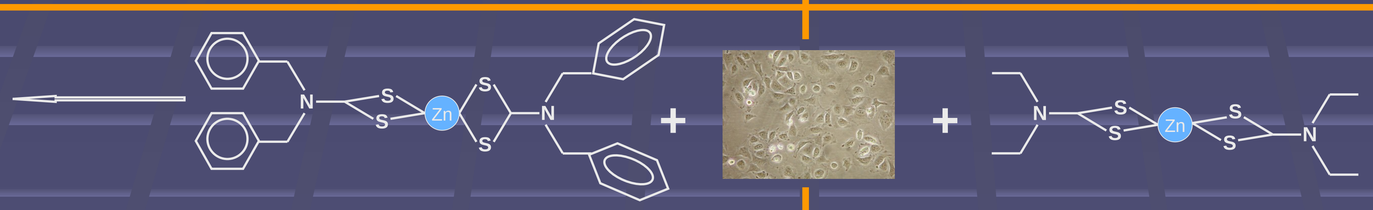


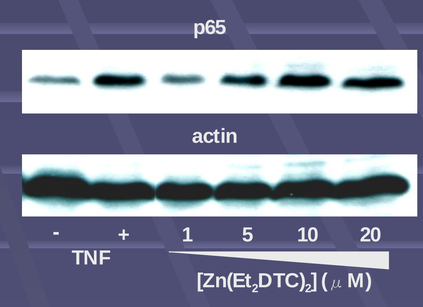
NF-kB & anticancer drugs:

Nakanishi & Toi *Nat Rev Cancer* 2005
cf. Ho et al. *Cancer Res* 2005 (!)





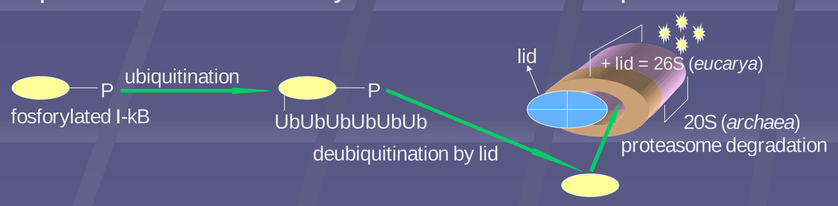




HeLa cells were treated in serum free medium for 30 min with:
b) tumor necrosis factor (TNF), NF-kB-activating cytokine, and $[Zn(Bz_2DTC)_2]$, b) $[Zn(Et_2DTC)_2]$
nuclear translocation of p65 was determined by western bloting

At the start of the story: pyrrolidine dithiocarbamate blocks proteolysis of I-kB and hence canonical NF-κB pathway both *in vitro* (Henkel et al. *Nature* 1993) and *in vivo* (Liu et al. *Mol Pharmacol* 1999). Why? These two ideas result from key studies: 1) the I-kB can be stabilized through proteasome inhibition or 2) via a blockage of ubiquitination.

Proteasome inhibitors: dithiocarbamate complexes, formed by reaction with Zn(II) and Cu(II) in medium, can enter the cell and inhibit proteasome (Kim et al. *Exp Cell Res* 2004; Chen et al. *Cancer Res* 2006). They inhibit its chymotrypsin-like activity (Milacic et al. *Cancer Res* 2006) as well as bortezomib, potent anticancer drug (Adams *Cancer Cell* 2004). Zn(II) + dimer of diethyldithiocarbamate (disulfiram) were successfully used for clinical remission in patient with metastatic ocular melanoma (Brar et al. *Mol Cancer Ther* 2004). What is a likely mechanism of proteasome inhibition by dithiocarbamate complexes?

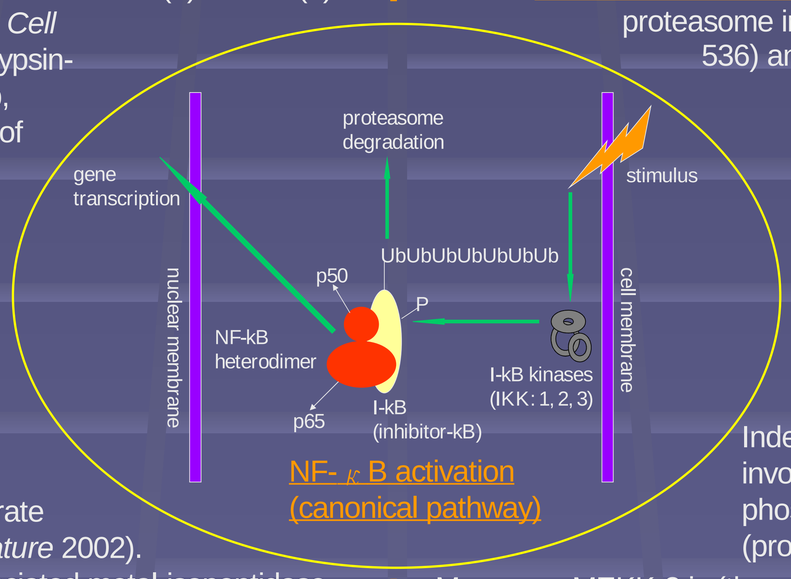


The lid subunit POH1 (Rpn11 in yeast) is responsible for substrate deubiquitination during proteasomal degradation (Yao et al. *Nature* 2002). This protein contains highly conserved Jab1/MPN domain-associated metal-isopeptidase (JAMM) motif, which is sensitive to metal chelators (Verma et al. *Science* 2002).

SCF inhibitors: pyrrolidine dithiocarbamate inhibits I-kB ubiquitin ligase in cell-free system (Hayakawa et al. *EMBO J* 2003). This ligase belongs to Skp-1/Cul/F box (SCF) family and is regulated by „deneddylation" of Cul1 subunit. Such event requires the isopeptidase activity of CSN5 subunit of the COP9 signalosome (Cope et al. *BMC Biochem* 2006; *cf.* Schweitzer et al. *EMBO J* 2007). CSN5 contains JAMM motif, sensitive to metals as well as metal chelators (Cope et al. *Science* 2002). See also Cvek & Dvorak *Curr Pharm Des* 2007 in press

Future directions: most recently, Milennium Pharmaceuticals researchers have reported JAMM motif of POH1 as therapeutic drug target for cancer (Gallery et al. *Mol Cancer Ther* 2007), so there are following challenges:

- extract the general principles of proteasome or CSN5 inhibition from recent studies
- design of new proteasome or CSN5 inhibitors for cancer therapy (the collaboration with B. A. Karmanos Cancer Institute Detriot USA and Department of Chemistry MU Brno)
- molecular structure of JAMM motif is known (Ambroggio et al. *PLoS Biology* 2004) and hence we can model (*in silico*) its inhibitors (collaboration wanted)



At the start of the story: dithiocarbamates are well known activators of AP-1 and AP-1-dependent gene induction both *in vitro* and *in vivo* (Meyer et al. *EMBO J* 1993; Borrello et al. *Arch Biochem Biophys* 1997 & *Biochem Biophys Res Commun* 1996). This capability can be linked to proteasome inhibition and proteasome-independent NF-kB pathway.

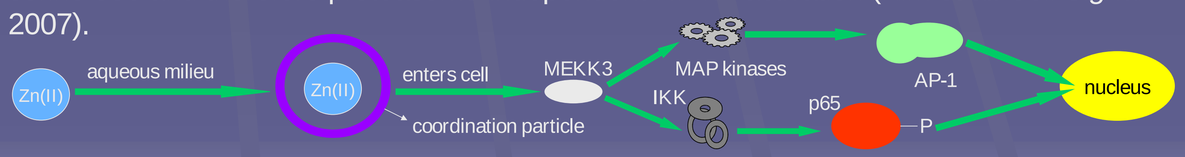
Proteasome inhibitors can activate NF-kB & AP-1: bortezomib and MG132, widely used proteasome inhibitors, trigger IKK1/2 mediated p65 phosphorylation (at serine 536) and activation simultaneously with I-kB degradation (Dolcet et al. *J Biol Chem* 2006). They, as well as pyrrolidine dithiocarbamate (by AP-1 activation: Hartsfield et al. *FASEB J* 1998), up-regulate heme oxygenase-1 gene. This effect of various proteasome inhibitors is NF-kB inhibition-independent and is mediated by p38/AP-1 pathway (Wu et al. *Biochem J* 2004).

Proteasome-independent „pathway X" : phosphorylated p65 (on serine 536) is not associated with I-kB and p50, hence its activation is totally proteasome-independent (Sasaki et al. *J Biol Chem* 2005). This defines new NF-kB pathway.

Indeed, signaling to NF-κB can be MEKK3-mediated: this pathway involves IKK3 phosphorylation and IKK1 activation, resulting in p65 phosphorylation and subsequent I-kB release from NF-kB without I-kB (proteasomal) degradation (Yao et al. *J Biol Chem* 2007).

Moreover, MEKK-3 is (through mitogen activated, = MAP, kinases) involved also in AP-1 activation (Xu et al. *J Biol Chem* 2004; Lee et al. *Mol Cell Biol* 2003).

Zn(II) activates „pathway X" : 1. zinc(II) induces AP-1 through MAP kinases (Kim et al. *Am J Physiol Lung Cell Mol Physiol* 2006). 2. zinc(II) exposure causes p65 phosphorylation on serine 536 and therefore proteasome-independent NF-kB activation (Kim et al. *Cell Signal* 2007).



Future directions: current anticancer research is focused on NF-kB, AP-1 (Mariani et al. *Cancer Cell* 2007), and IKK (Luo et al. *Nature* 2007), so we need

- to extract the general principles of NF-kB activation, signaling, and cross-talk with AP-1 from recent studies
- to answer these questions: what type of coordination sphere and why does trigger NF-kB and AP-1 pathways? what implications are there for anticancer therapy?
- to know in what form and how do dithiocarbamate coordination compounds enter the cell (collaboration wanted)

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