Prevention of Cr(VI)-induced Carcinogenesis by natural compounds

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Two stages of metal carcinogenesis

(a) Malignant cell transformation (first stage) from normal cells to malignantly transformed cells.

(b) Tumorigenesis (second stage) from transformed cells to tumor.

This prevention:

Part 1. Mechanisms at both stages

Part 2. Mechanism-based prevention

Part I. First stage of Cr(VI)-carcinogenesis

From normal cells to malignant transformed cells

Reactive oxygen species (ROS) play a major role in this stage (ROS are oncogenic, bad).

1. Mechanism of Cr(VI)-induced ROS generation

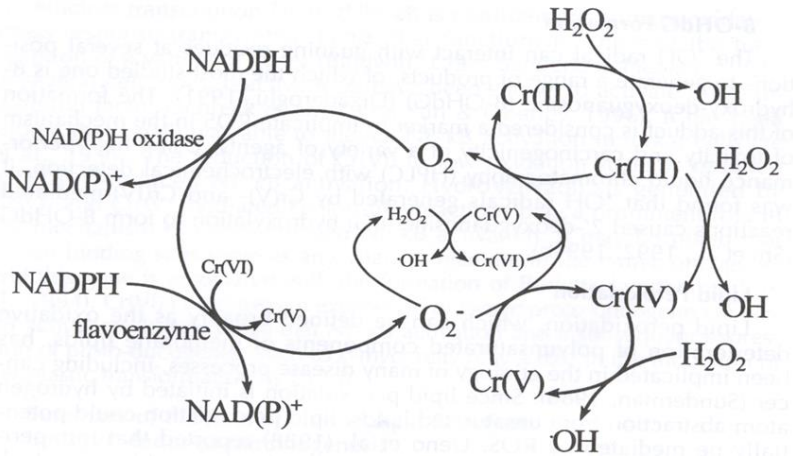
(1) Cr(VI)-reduction by cellular reducing agents

$$Cr^{6+} + O_2^{\cdot -} \rightarrow Cr^{5+} + O_2 \quad [1]$$

$$Cr^{5+} + H_2O_2 \rightarrow Cr^{6+} + OH^- + \cdot OH \quad [2]$$

[1] + [2]

$$H_2O_2 + O_2^{\cdot -} \xrightarrow{Cr^{6+}/\ Cr^{5+}} {}^{\cdot}OH + \cdot OH + O_2$$



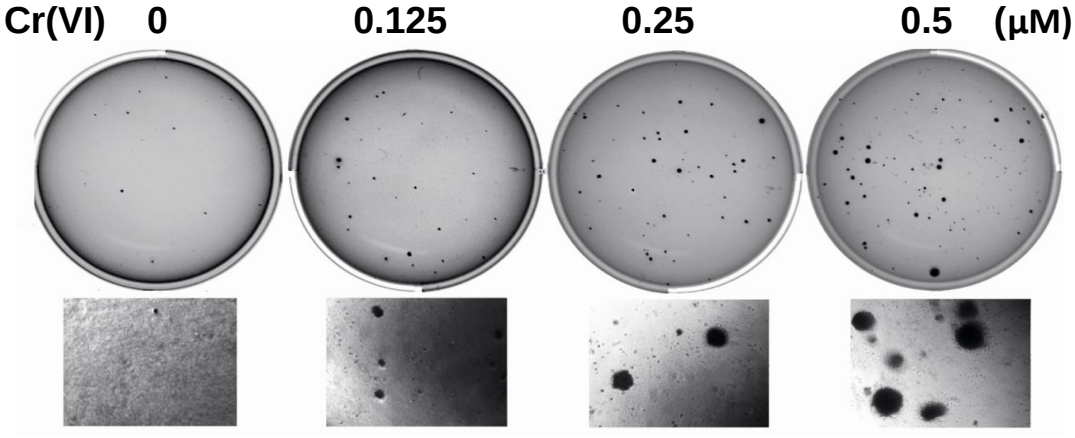
Lee, J.C., Son, Y.O., Pratheeshkumar, P., and Shi, X. *Free Radical Biology and Medicine* 53, 742-757, 2012.

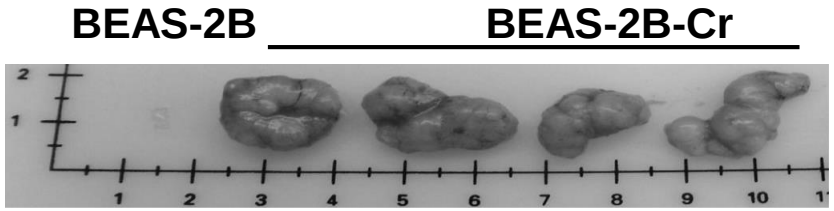
(2) Cells stimulated by Cr(VI)

Cr(VI) → Cdc42 → p47^{phox} and p67^{phox} →
NADPH oxidase → ROS (O_2 → $O_2^{\bullet -}$ → H_2O_2 → $^{\bullet}OH$)

2. Cr(VI) induces cell malignant transformation through ROS

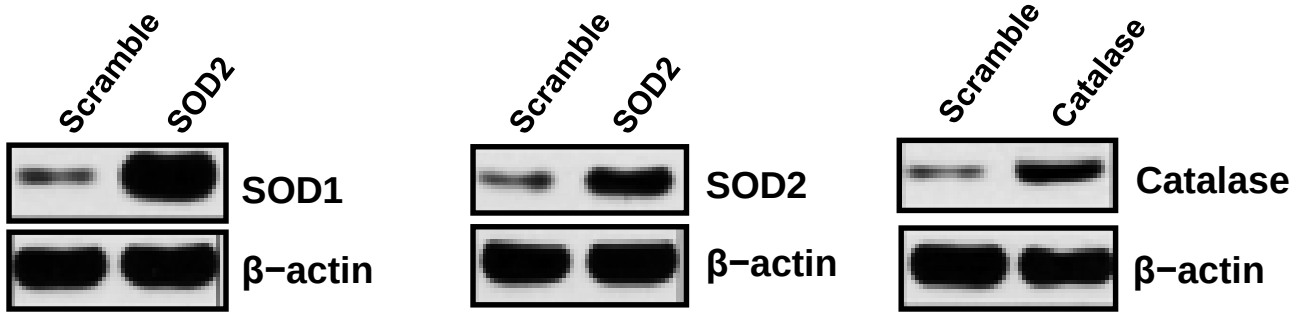
Cr(VI) induces cell transformation of BEAS-2B cells

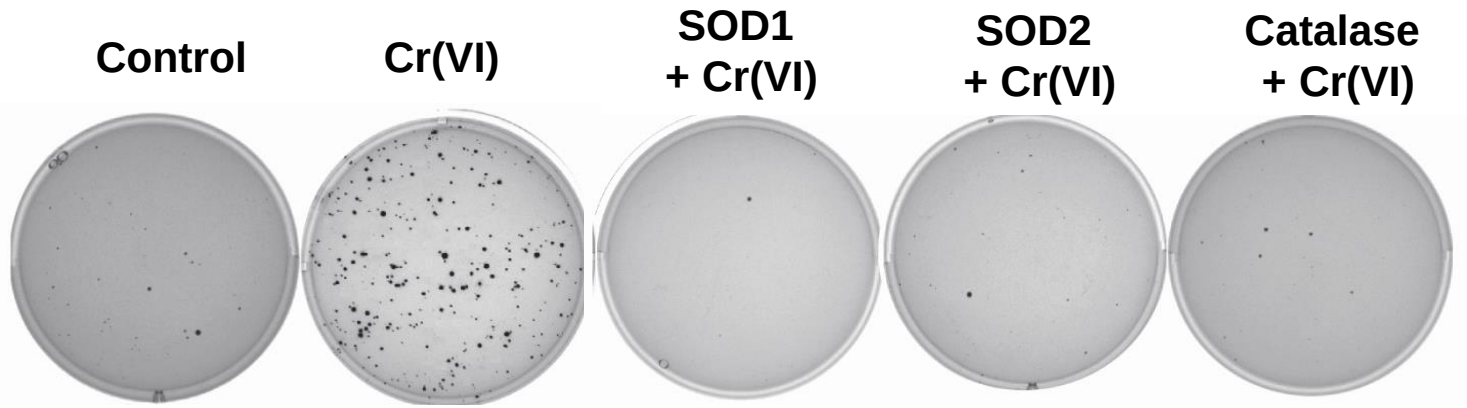




Human lung bronchial epithelial cells (BEAS-2B)

Inhibition of ROS reduces cell transformation induced by Cr(VI)





SOD: superoxide dismutase
Catalase: hydrogen peroxide

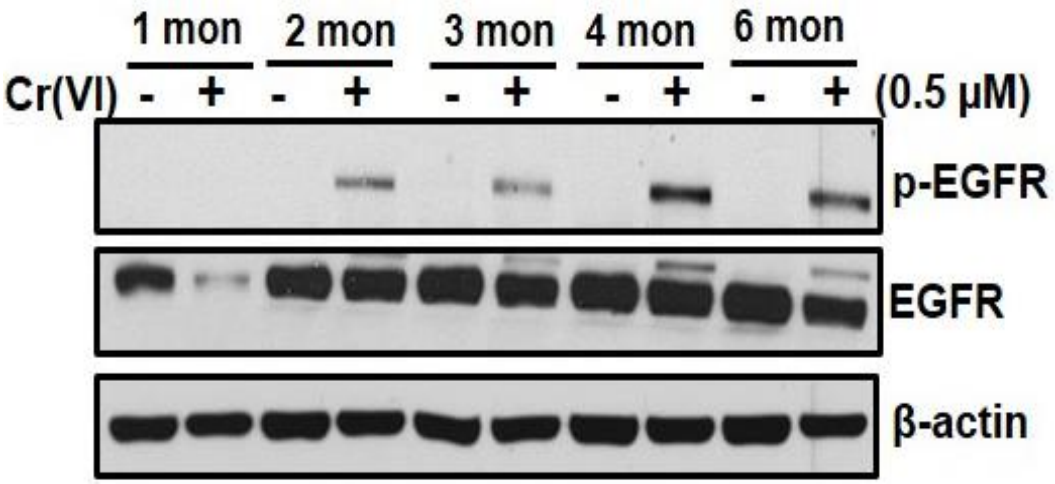
Part II. Second stage of Cr(VI) carcinogenesis

Tumorigenesis: from Cr(VI)-transformed cells to tumor

ROS are anti-oncogenic (good) in this stage.

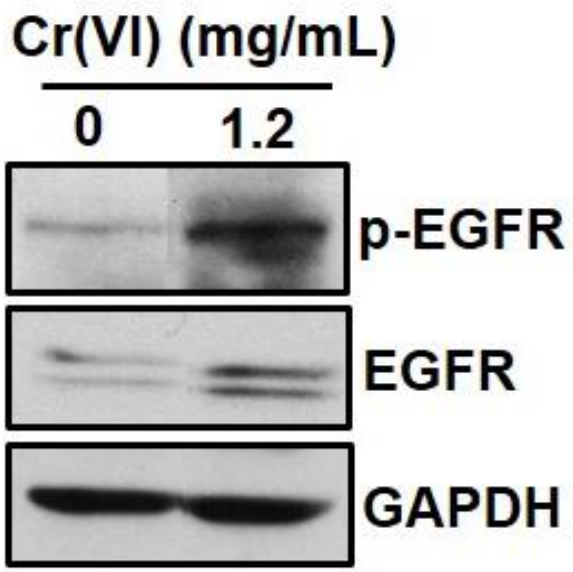
Prevention strategy: increase ROS by natural compounds

1. EGFR activation during Cr(VI)-induced cell transformation

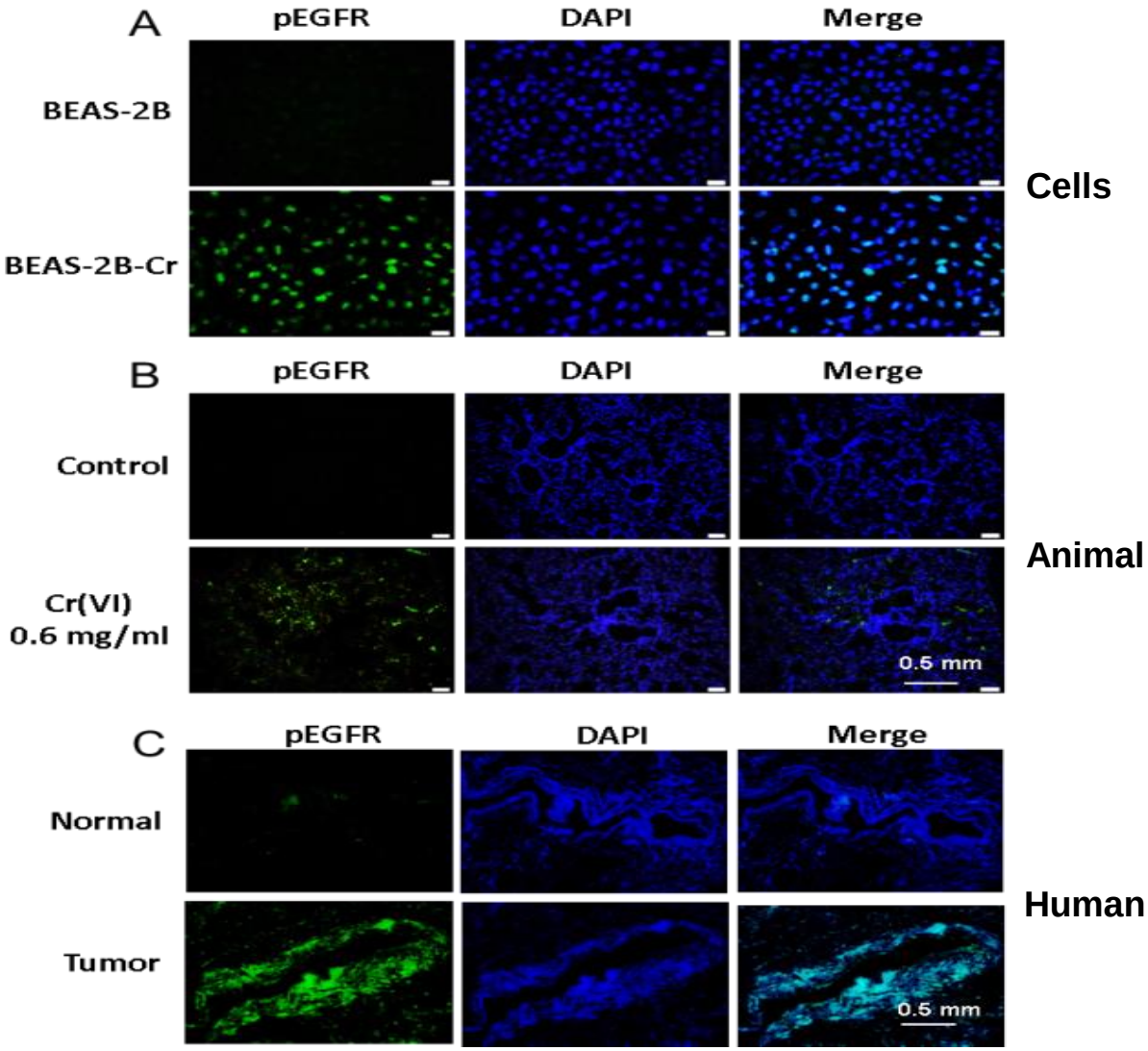


Epidermal growth factor receptor (EGFR)
Human lung bronchial epithelial cells (BEAS-2B)

EGFR activation in lung tissue of animal chronically exposed to Cr(VI) for 20 weeks

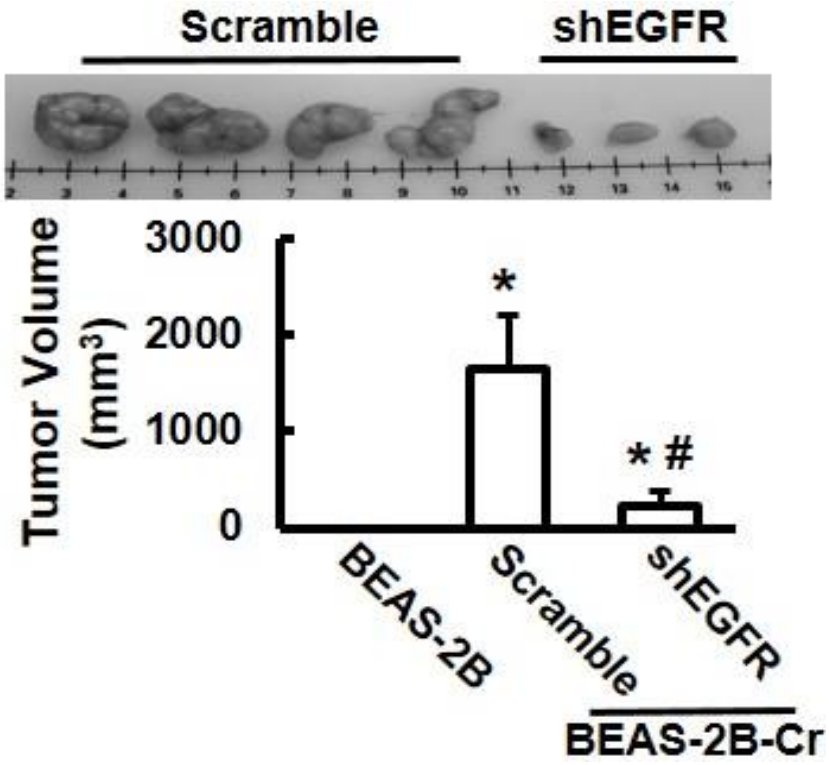


Activation of EGFR in Cr(VI)-transformed cells, in lung tissue of animal exposed to Cr(VI) for 20 weeks, and in lung tumor tissue from a non-smoking worker exposed to Cr(VI) for 19 years



Human samples were from Kazuya Kondo, University of Tokushima, Japan

Role of EGFR in tumorigenesis of Cr(VI)-transformed cells



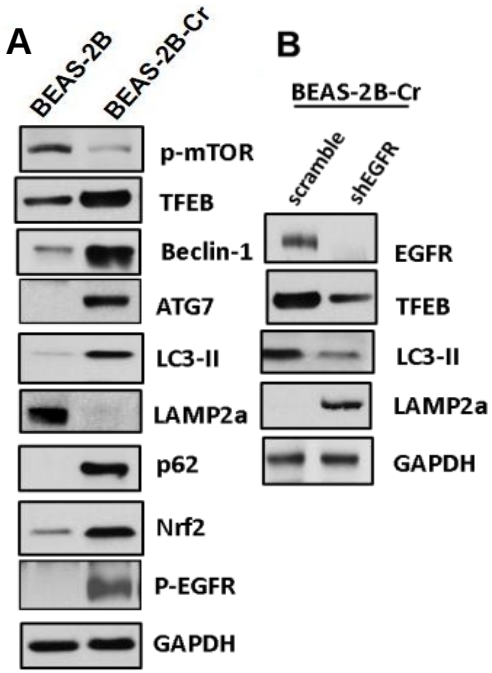
Epidermal growth factor receptor (EGFR)
Human lung bronchial epithelial cells (BEAS-2B)

2. EGFR initiates autophagy and causes autophagy deficiency (or defect) in Cr(VI)-transformed cells

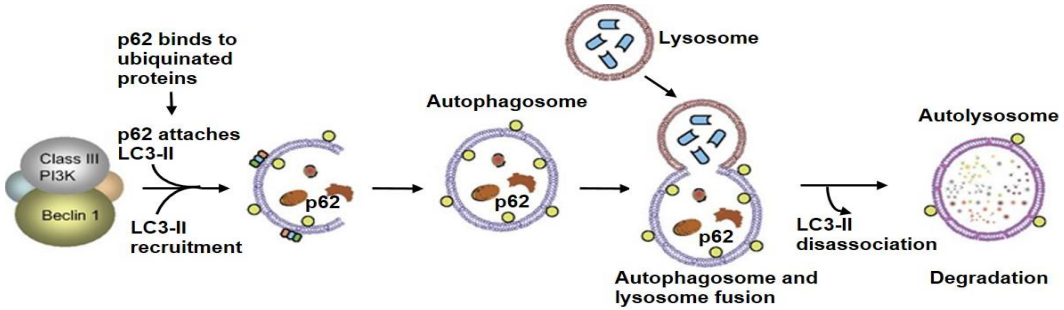
Autophagy is a survival-promoting pathway that captures, degrades, and recycles intracellular proteins and organelles in lysosomes.

1. Controlled digestion of damaged organelles within a cell.
2. The maintenance of bodily nutrition by the metabolic breakdown of some bodily tissues.

Alternations of autophagy regulative proteins by EGFR



Schematic diagram of the steps of autophagy

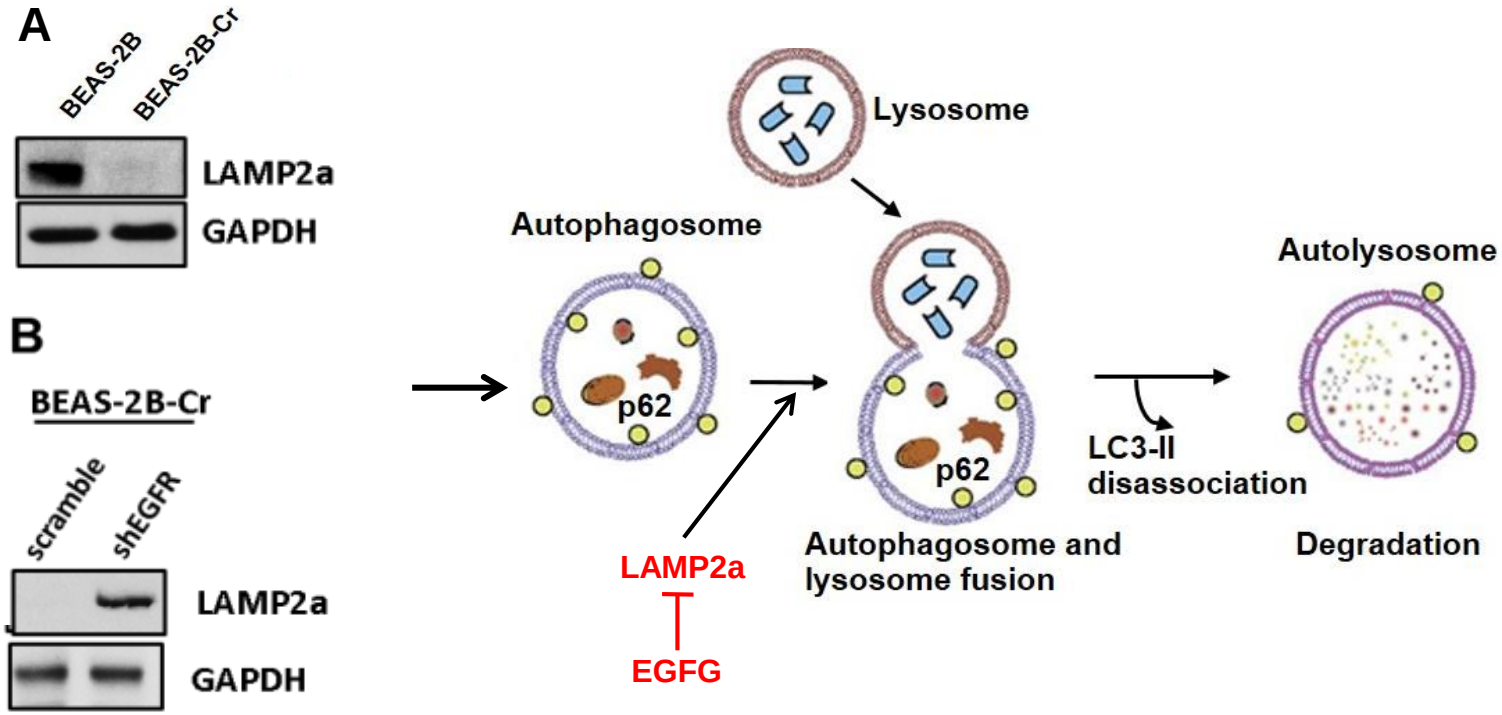


In Cr(VI)-transformed cells:

1. Autophagy is initiated (mTOR down and TFEB, Beclin-1, Atg7, and LC3-II up)
2. Autophagosomes are not fused with lysosomes (LAMP2a down and p62 up)
3. EGFR activation initiates autophagy and stops at the fuse stage

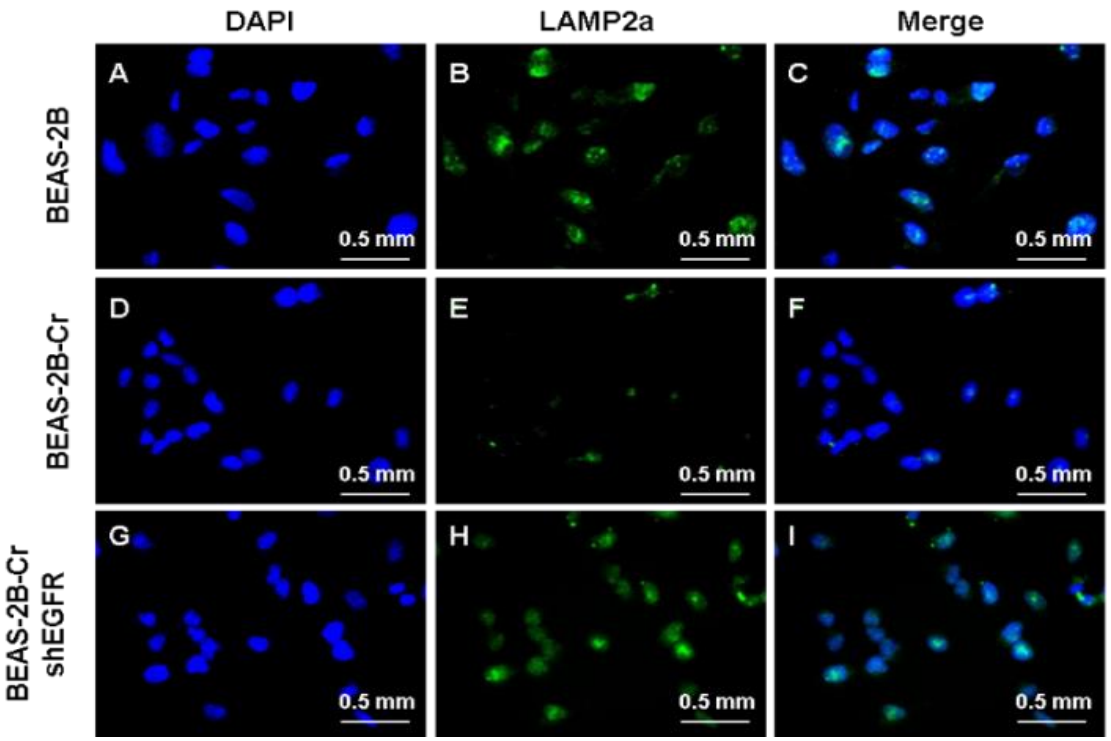
Transcription Factor EB (TFEB)
Microtubule-associated protein 1A/1B-light chain 3 (LC3)
Lysosome-associated membrane protein 2 (LAMP2)

EGFR down-regulates LAMP2a

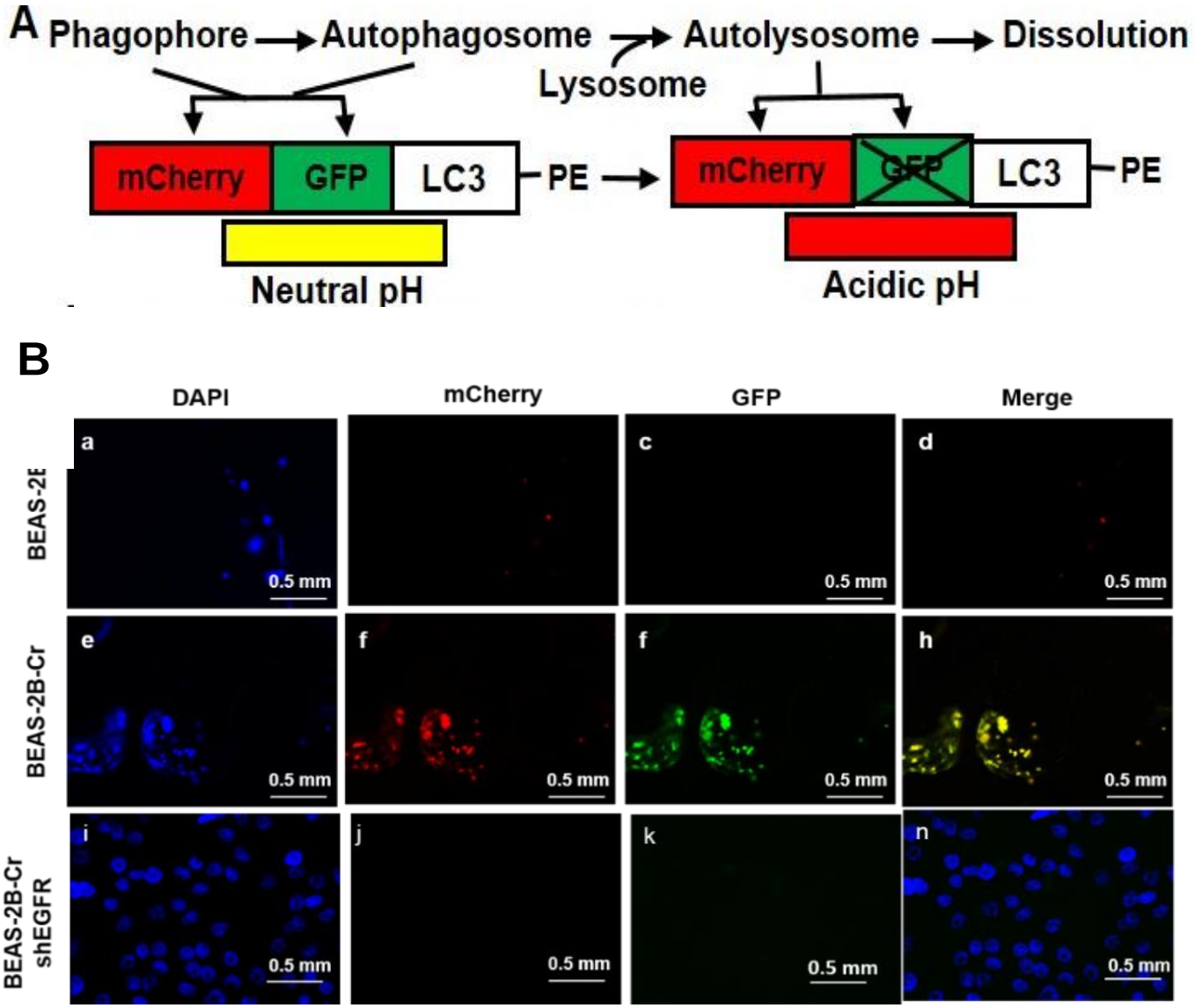


Note:
The down-regulation of LAMP2a by EGFR integrates EGFR signaling with autophagy pathway.

EGFR down-regulates LAMP2a



EGFR initiates autophagy and causes autophagy deficiency

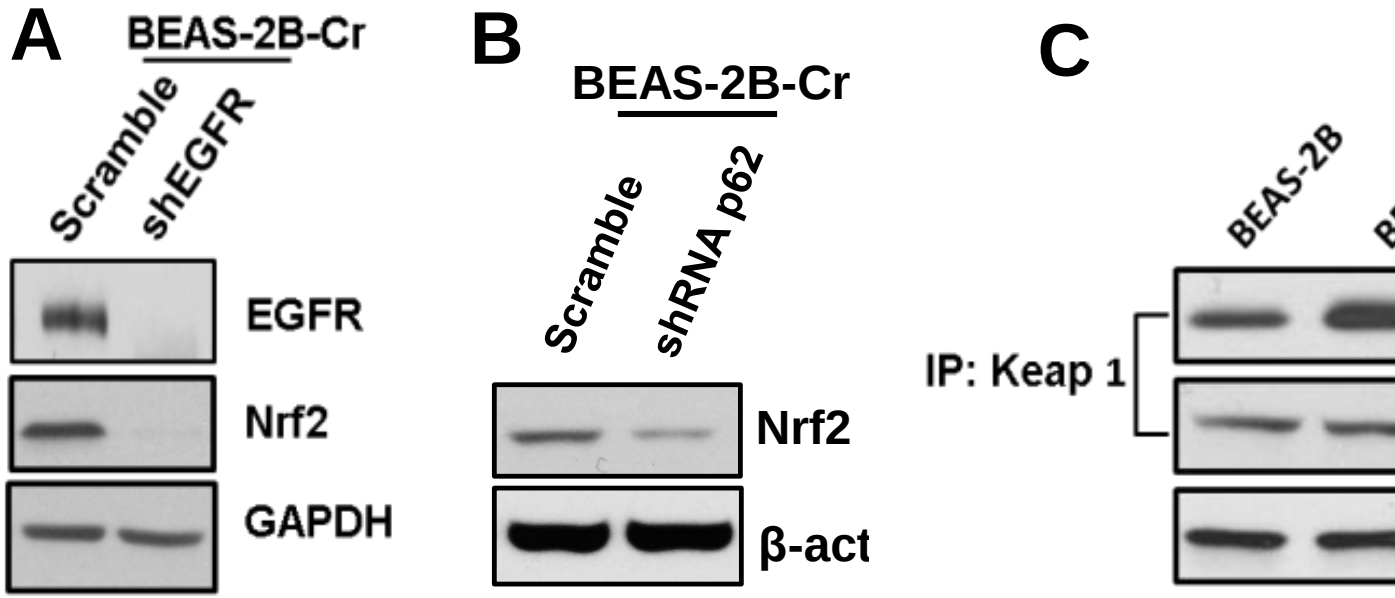


3. Consequence of autophagy deficiency:

(a) Accumulation of p62
(b) Constitutive activation of Nrf2
(c) Increase in antioxidant proteins
(d) Decrease in ROS
(e) Increase in anti-apoptosis proteins, Bcl-2 and Bcl-XL
(f) Angiogenesis
(g) Inflammation

ROS are anti-oncogenic (good) in transformed ells.

p62 causes constitutive Nrf2 activation



pEGFR → Autophagy deficiency →

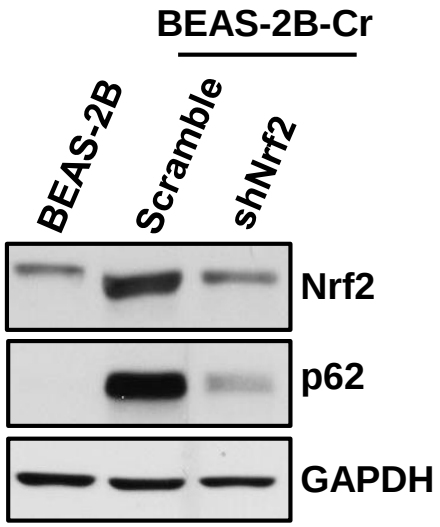
p62 → Keap-1 → Nrf2 (constitutive) ↑

↓

p62-Keap1

Nrf2 up-regulates p62 by positive feed-back

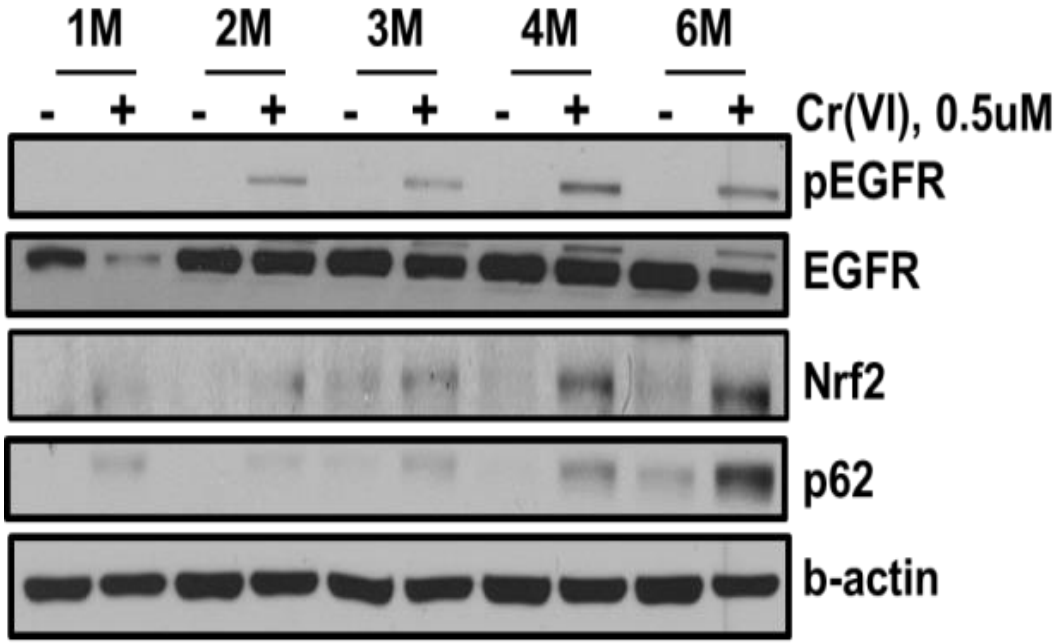
p62 ⇌ Nrf2



Nrf2 up-regulates p62 through binding of Nrf2 to the ARE site of p62 promoter

Data not shown:
Nrf2 binds to the ARE site of p62

Up-regulations of pEGFR, Nrf2, and p62 during Cr(VI)-induced cell transformation

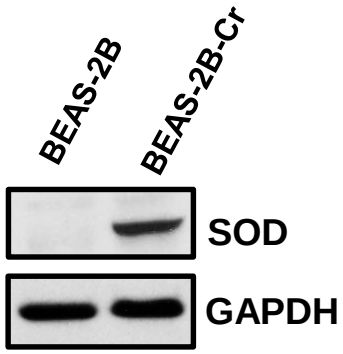


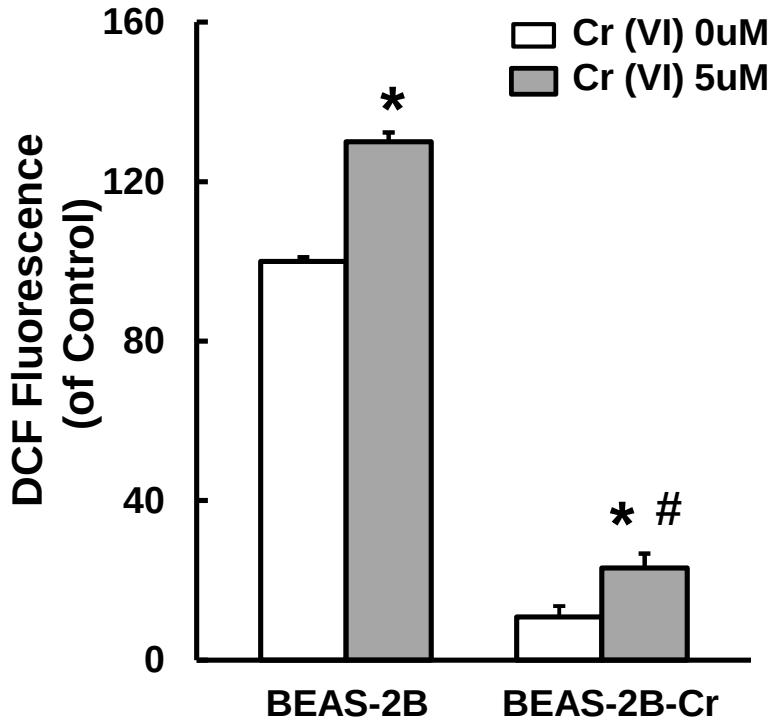
Nrf2 regulates its target proteins: antioxidant proteins and anti-apoptosis proteins

1. Nrf2 is able to bind to antioxidant response element (ARE) of antioxidant enzymes and Bcl-2 and Bcl-xL.

2. Constitutive activation of Nrf2 up-regulates various antioxidant enzymes (down regulation of ROS) and several anti-apoptotic proteins (Bcl-2 and Bcl-xL), leading to apoptosis resistance

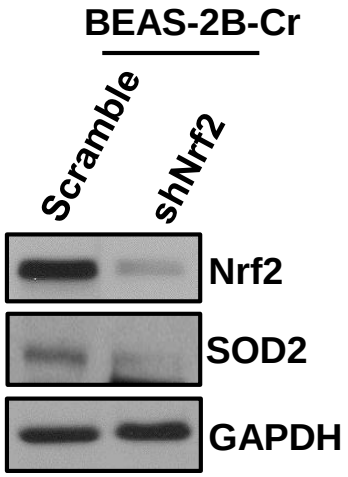
Increased antioxidant and reduced ROS (H_2O_2) generation in Cr(VI)-transformed cells

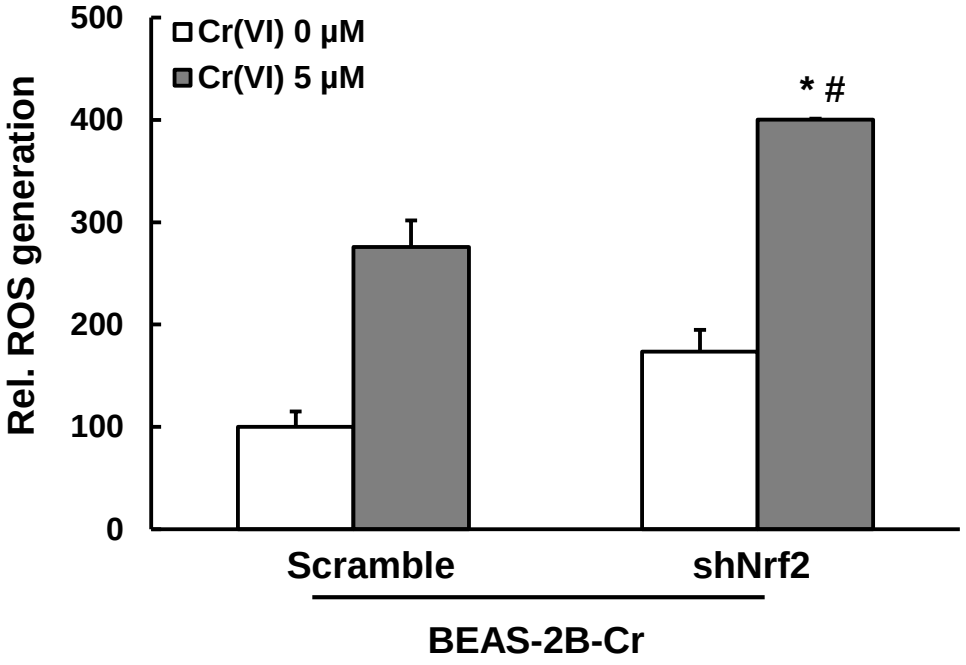




Data not shown:
Nrf2 binds to the ARE site of SOD
SOD: superoxide dismutase

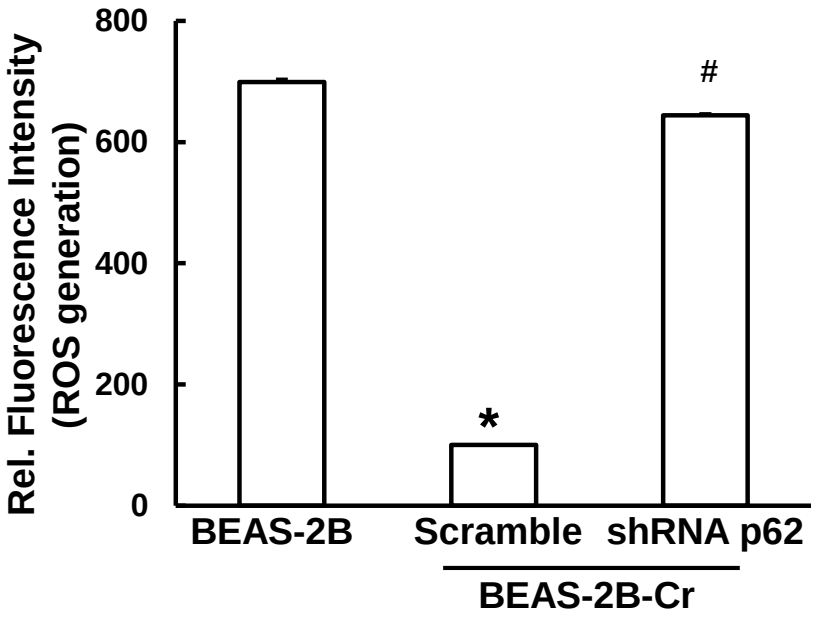
Inhibition of Nrf2 reduces SOD2 expression and increases ROS generation in Cr(VI)-transformed cells





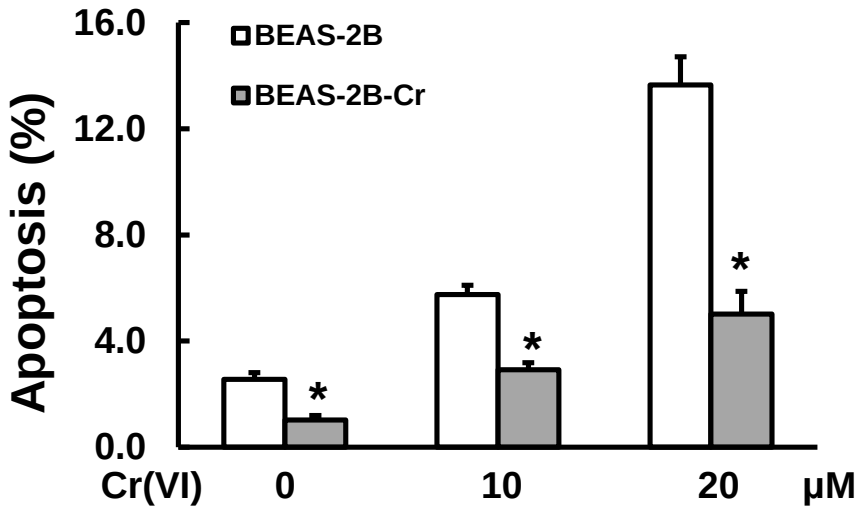
Nrf2 → antioxidant enzyme → ROS down

Inhibition of p62 increases ROS generation in Cr(VI)-transformed cells

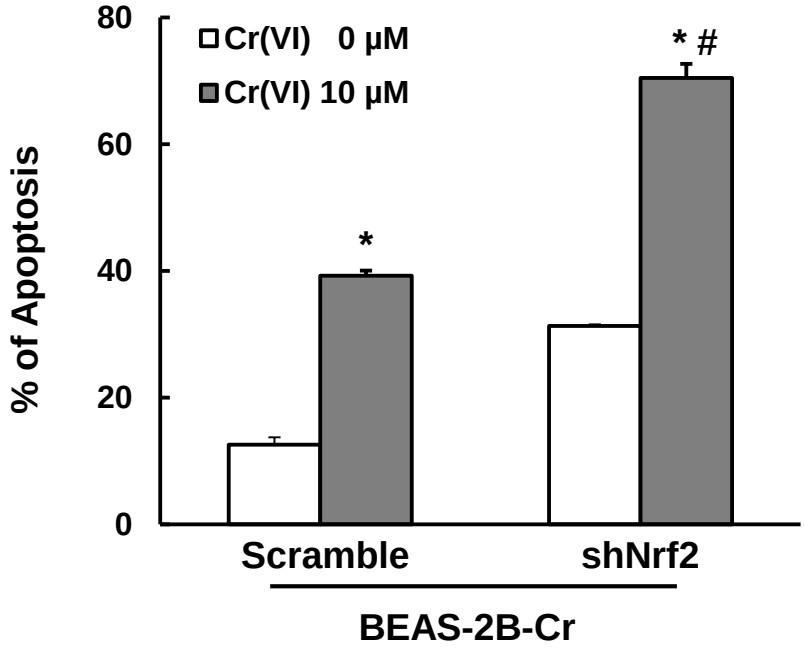


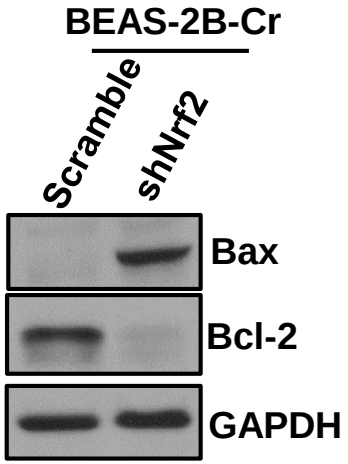
p62 → Nrf2 → antioxidant → ROS down

Apoptosis resistance of Cr(VI)-transformed cells



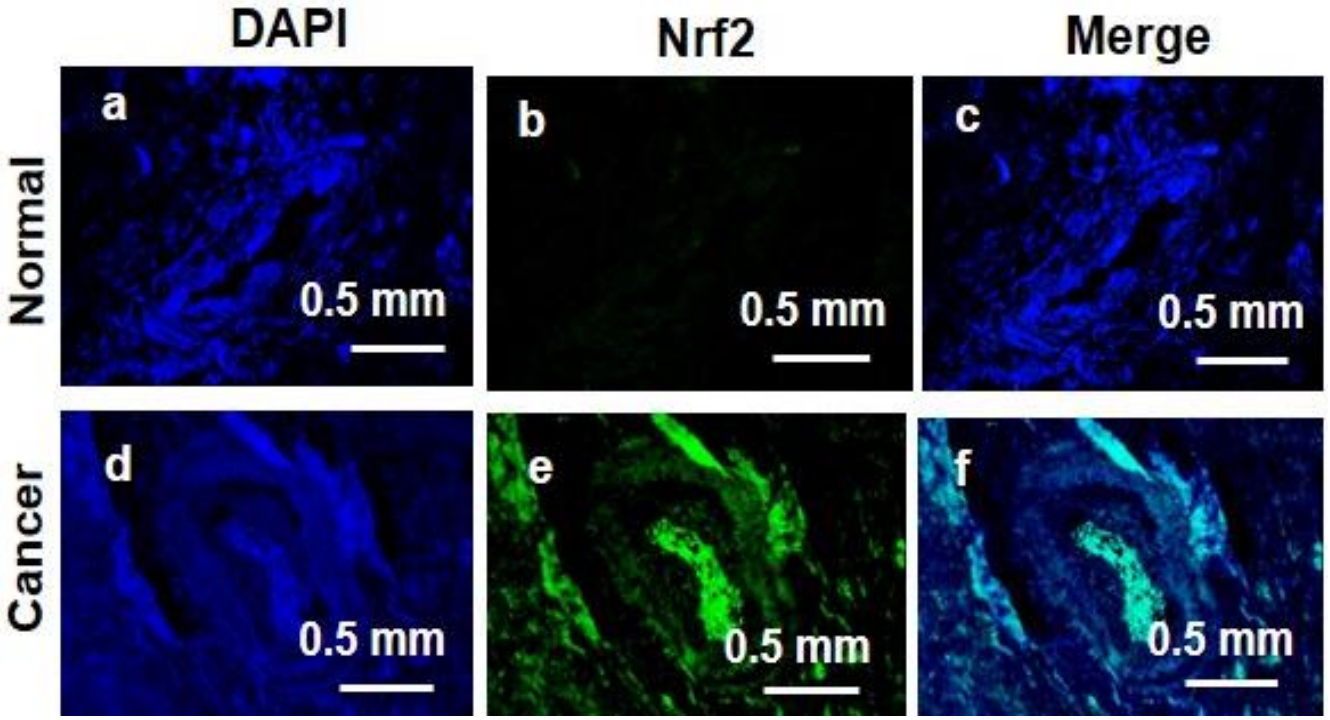
Inhibition of Nrf2 increases apoptosis in Cr(VI)-transformed cells



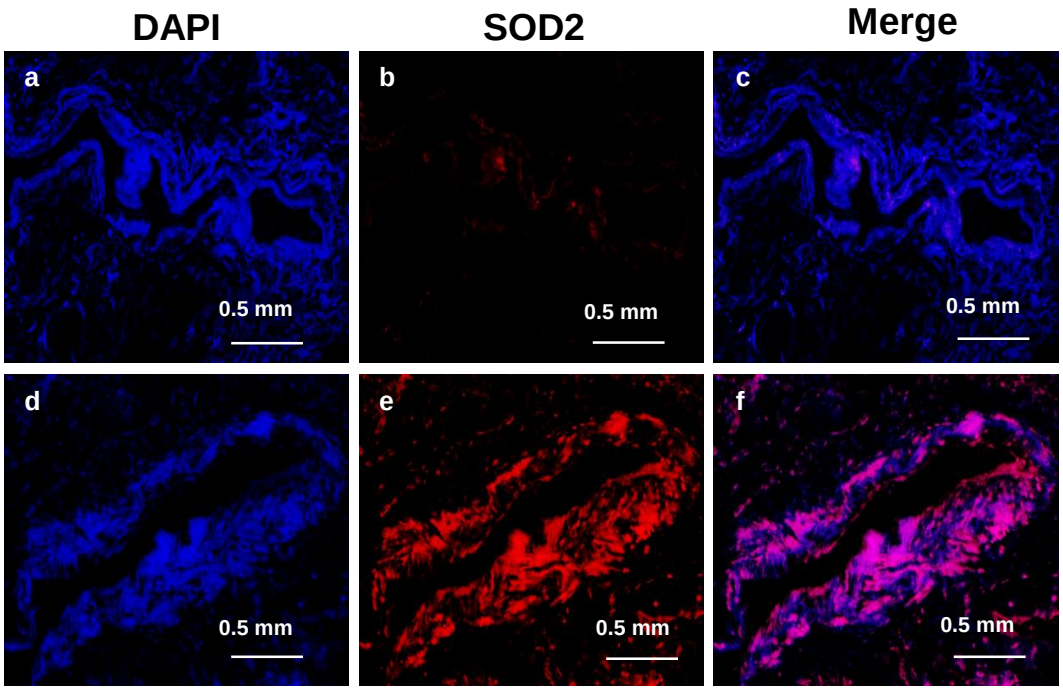


Data not shown:
Nrf2 binds to the ARE site of Bcl-2

Increased expression of Nrf2 in lung tumor tissue from a non-smoking worker at age of 62 exposed to Cr(VI) for 19 years.

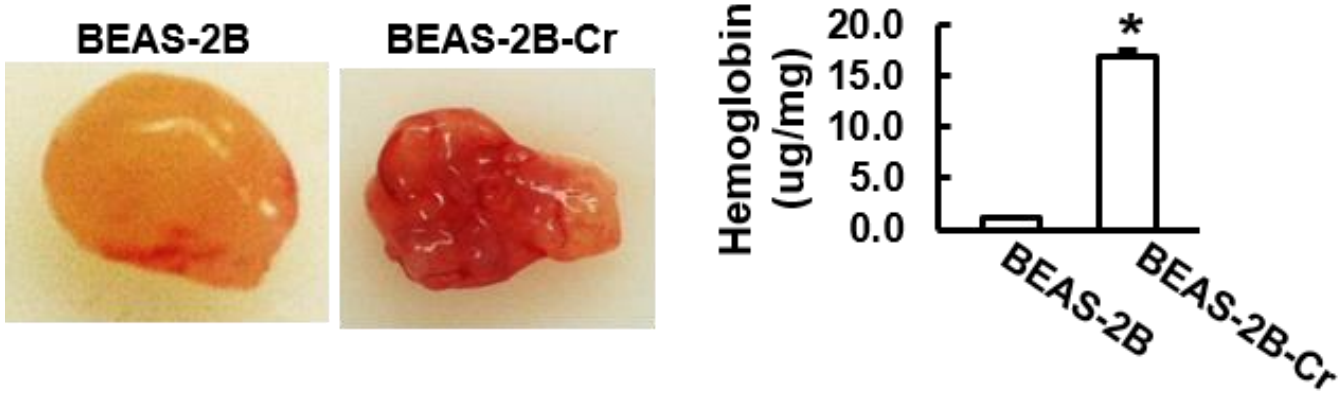


Increased SOD2 expression in the tissue of lung cancer patient exposed to Cr(VI)

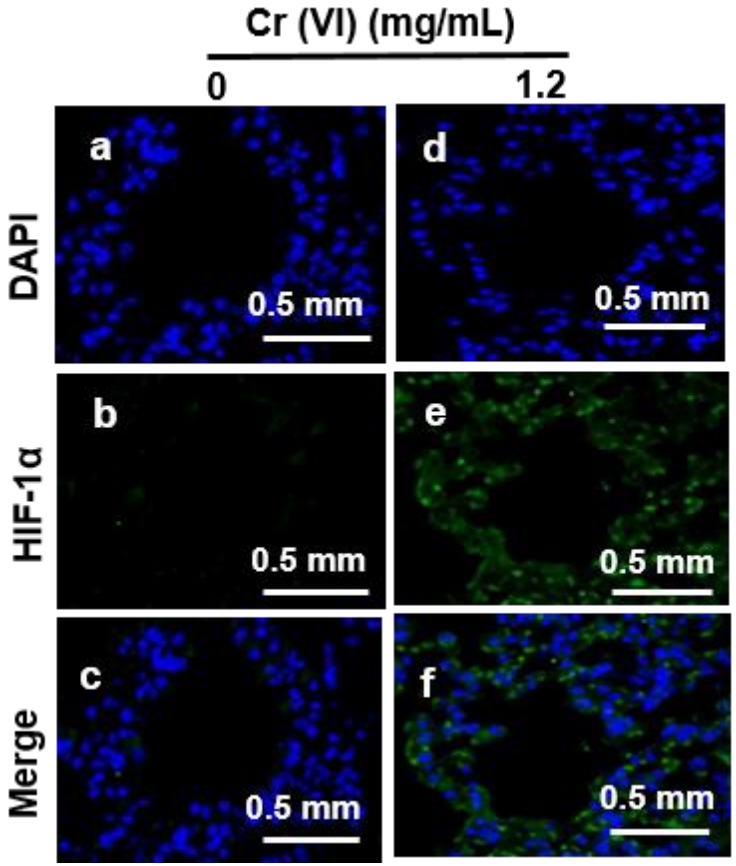


4. Consequence of autophagy deficiency: increase of HIF-1α and induction of angiogenesis

Cr(VI)-transformed cells induced angiogenesis in vivo: in vivo Matrigel plug assay

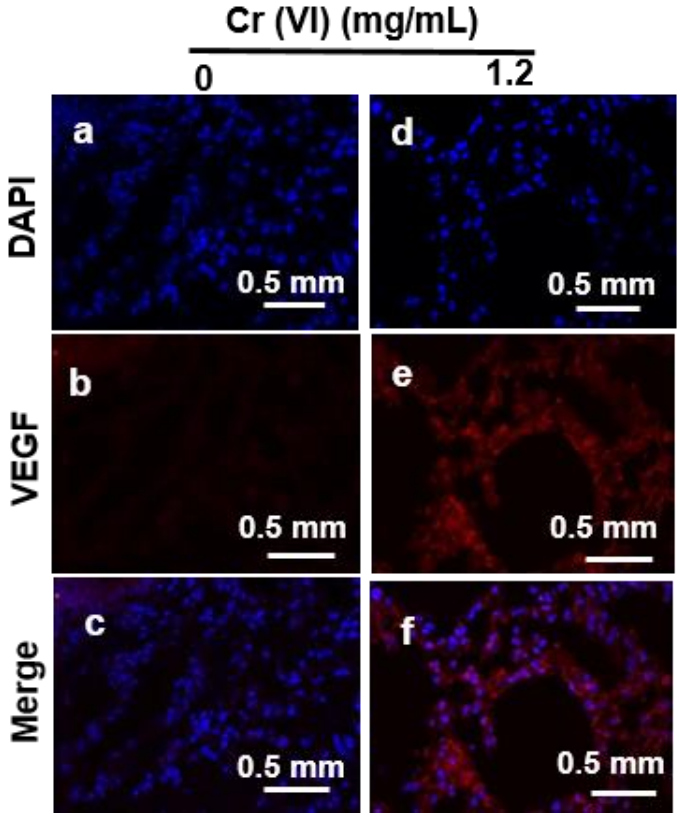


Expressions of HIF-1α in lung tissues from animals exposed to Cr(VI) for 12 weeks



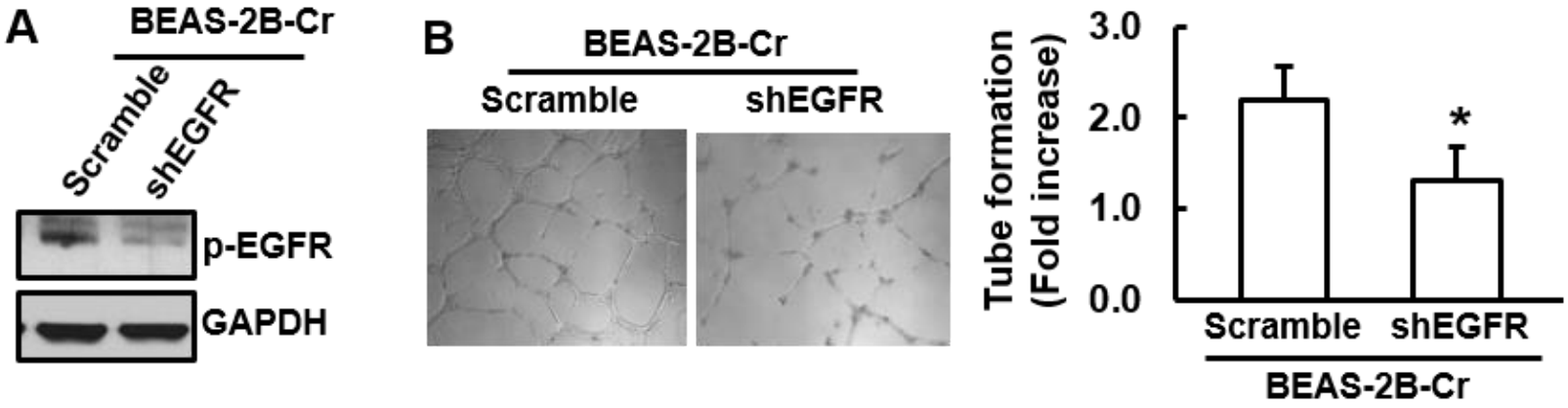
HIF-1α: Hypoxia-inducible factor 1α

Expressions of VEGF in lung tissues from animals exposed to Cr(VI) for 12 weeks

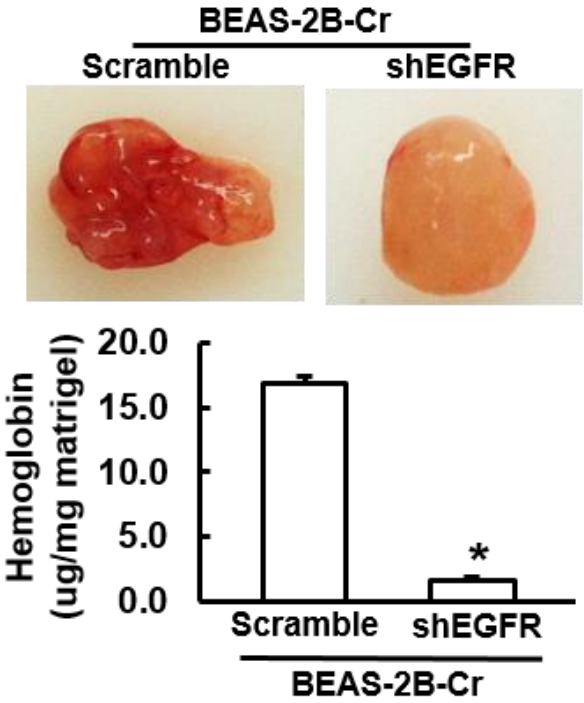


VEGF: vascular endothelial growth factor

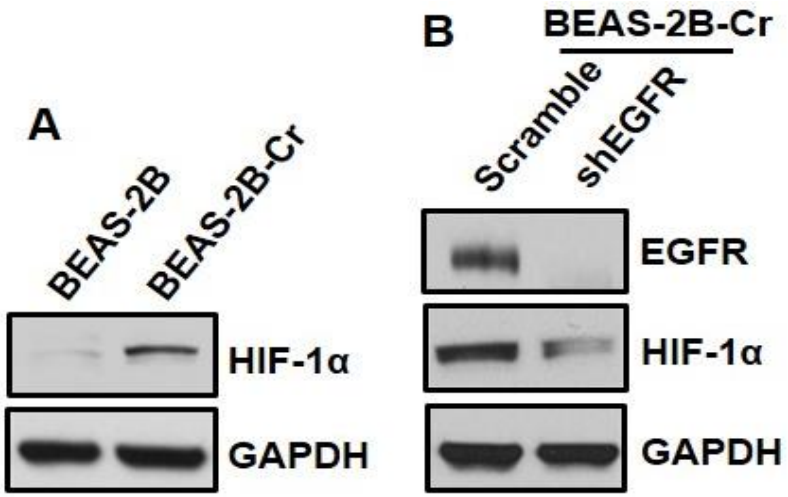
EGFR is required for angiogenesis



EGFR is required for angiogenesis (in vivo Matrigel plug assay)

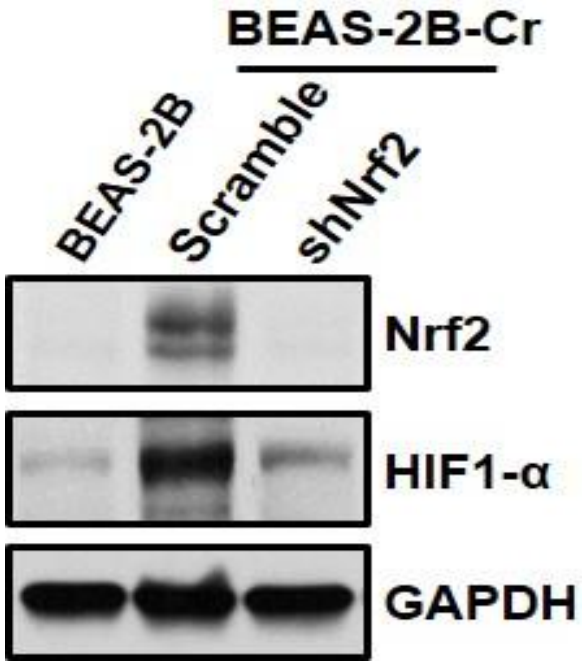


EGFR is in the up-stream of HIF-1α



pEGFR ⟶ HIF-1α

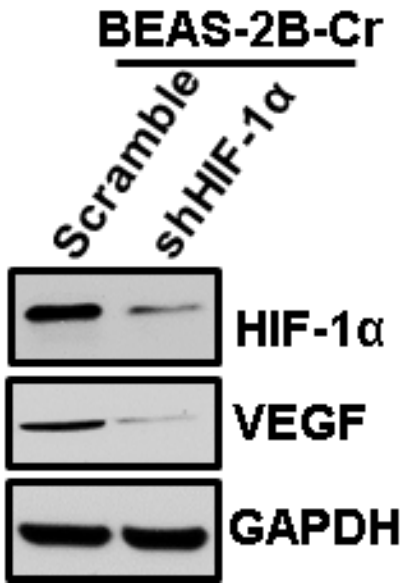
Nrf2 up-regulates HIF-1α



Nrf2 ⟶ HIF-1α

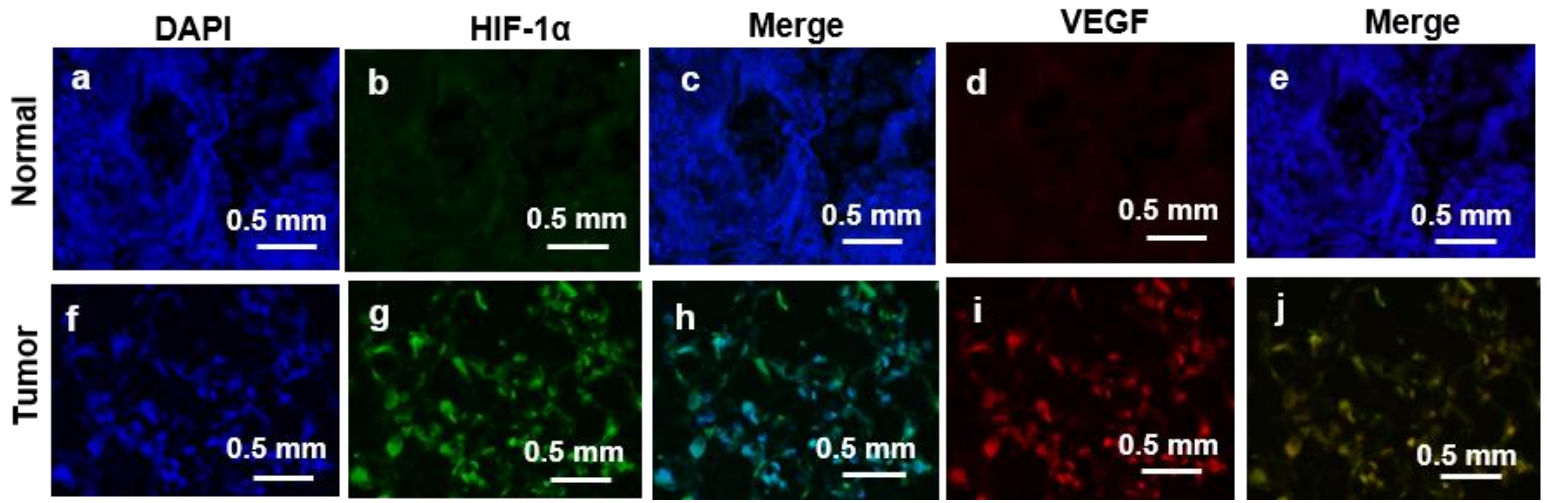
Hypoxia-inducible factor 1α (HIF-1α) is an important positive angiogenesis regulative protein.

HIF-1α up-regulates VEGF



HIF-1α ⟶ VEGF

Expressions of HIF-1α and VEGF in human lung tumor and its adjacent normal tissue from a worker exposed to Cr(VI) for 19 years with diagnosis of stage 1 squamous lung carcinoma



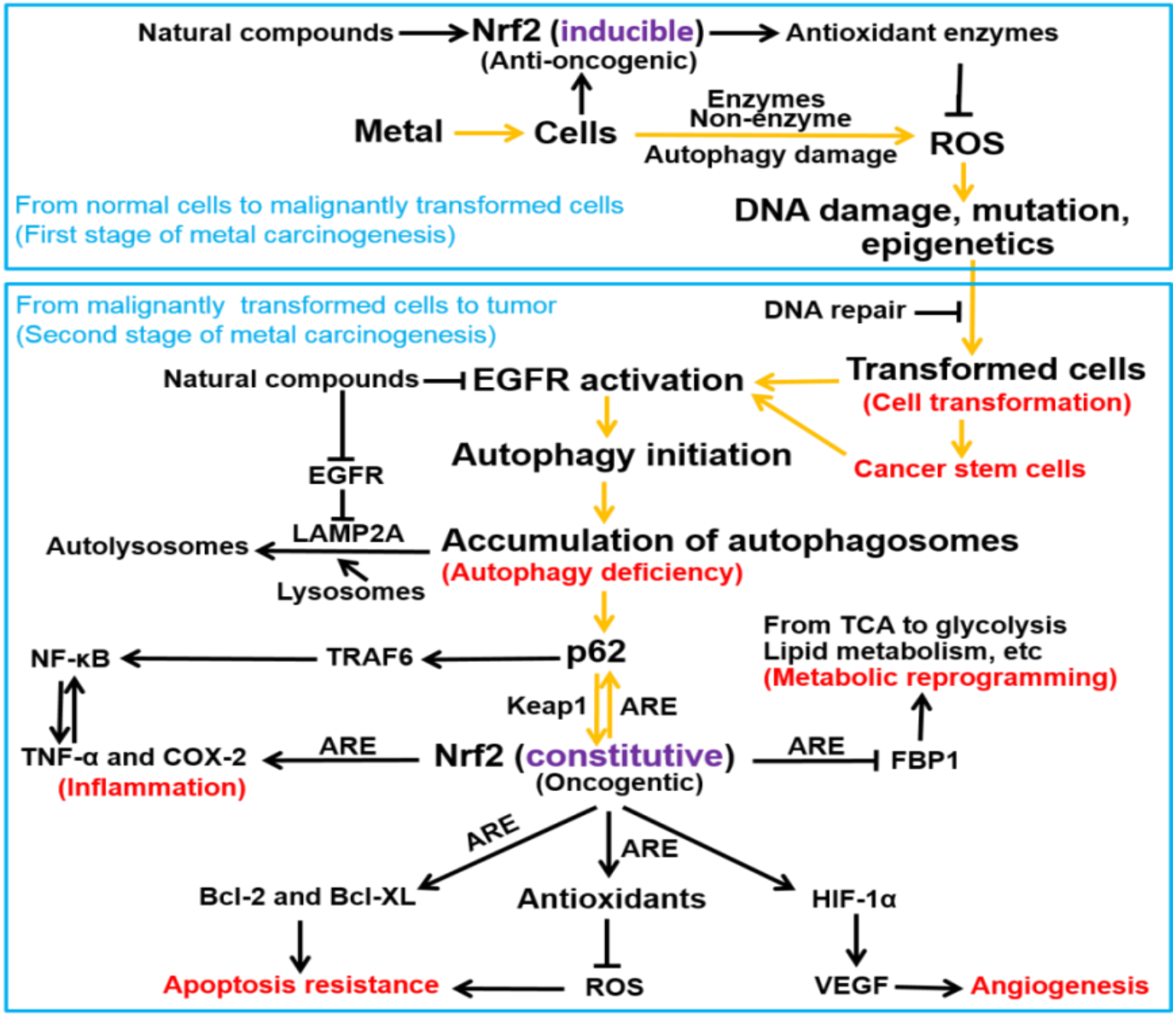
Angiogenesis of Cr(VI) transformed cells (summary)

(a) EGFR is required for angiogenesis
(b) Nrf2 up-regulates VEGF

pEGFR → → → Nrf2 → HIF-1α → VEGF

→ angiogenesis → tumorigenesis

Part III. Prevention of Cr(VI) carcinogenesis

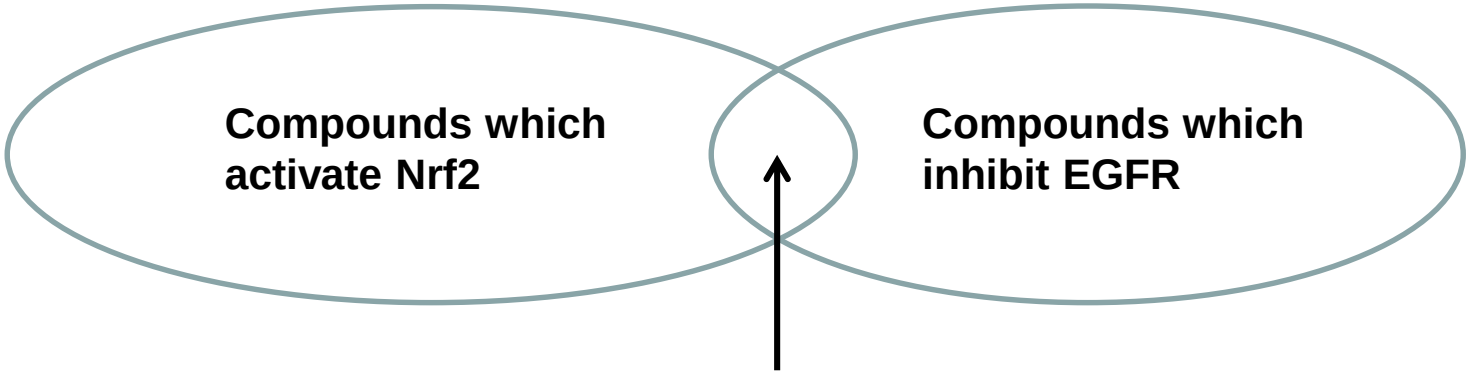

Natural compounds
Nrf2 (inducible)
(Anti-oncogenic)
Antioxidant enzymes
Metal
Cells
Enzymes
Non-enzyme
Autophagy damage
ROS
From normal cells to malignantly transformed cells
(First stage of metal carcinogenesis)
DNA damage, mutation, epigenetics
From malignantly transformed cells to tumor
(Second stage of metal carcinogenesis)
DNA repair
Natural compounds
EGFR activation
Transformed cells
(Cell transformation)
Autophagy initiation
Cancer stem cells
EGFR
LAMP2A
Autolysosomes
Lysosomes
Accumulation of autophagosomes
(Autophagy deficiency)
From TCA to glycolysis
Lipid metabolism, etc
(Metabolic reprogramming)
NF-κB
TRAF6
p62
Keap1
ARE
TNF-α and COX-2
(Inflammation)
ARE
Nrf2 (constitutive)
(Oncogentic)
ARE
FBP1
ARE
ARE
Bcl-2 and Bcl-XL
Antioxidants
HIF-1α
Apoptosis resistance
ROS
VEGF
Angiogenesis

Prevention of Cr(VI) carcinogenesis based on dual roles of Nrf2 using natural compounds

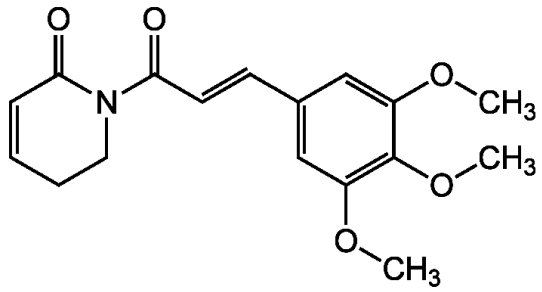
(a) In normal cells, induction of <u>inducible</u> Nrf2 decreases ROS, leading to inhibition of cell malignant transformation.

(b) In transformed cells, inhibition of <u>constitutive</u> Nrf2 decreases antioxidant enzymes, increases ROS, and restore apoptosis as well as decreased angiogenesis.

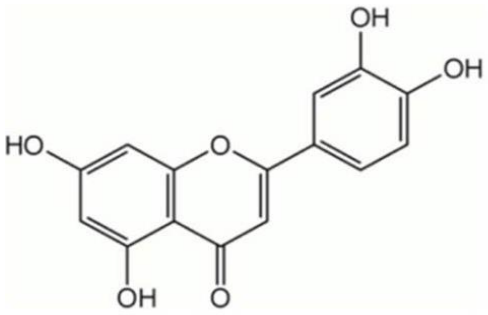
Strategy:
Induce Nrf2 in normal cells and reduce it in transformed cells



Select the ones which activate Nrf2 in normal cells and inhibits Nrf2 via inhibition of EGFR in transformed cells

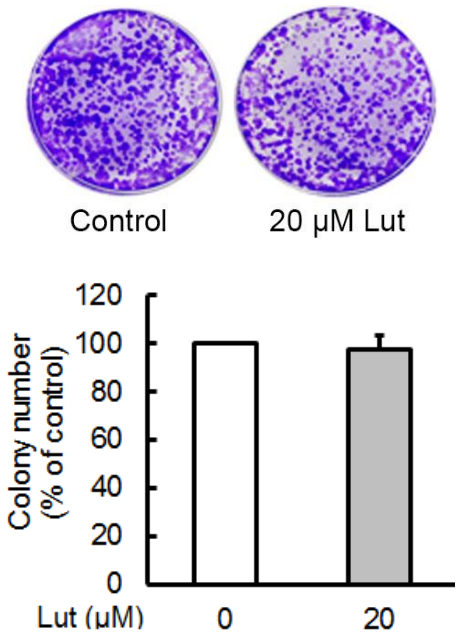


Piperlongumine (PL)



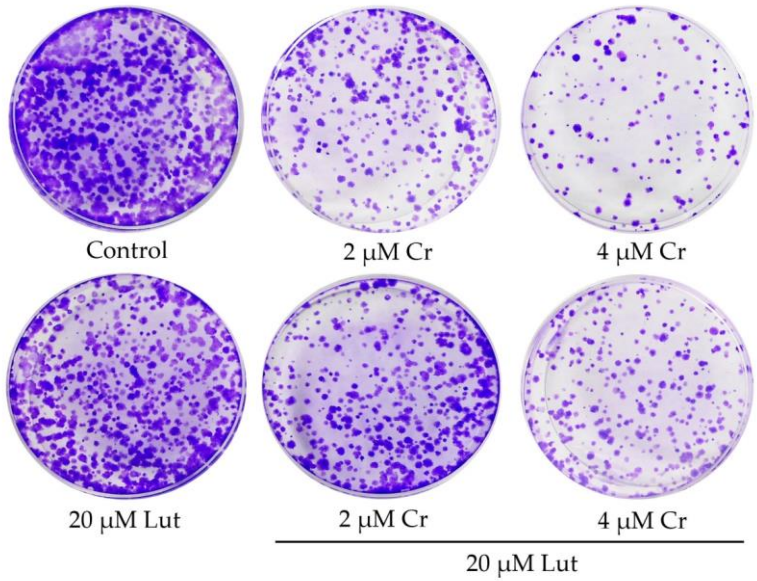
Luteolin (lut)

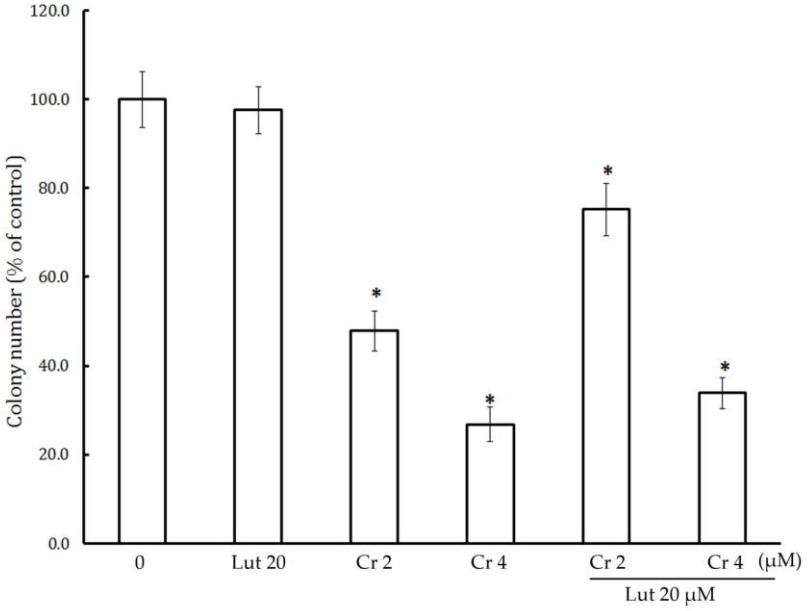
Luteolin does not exhibiy observable toxicity in normal cells



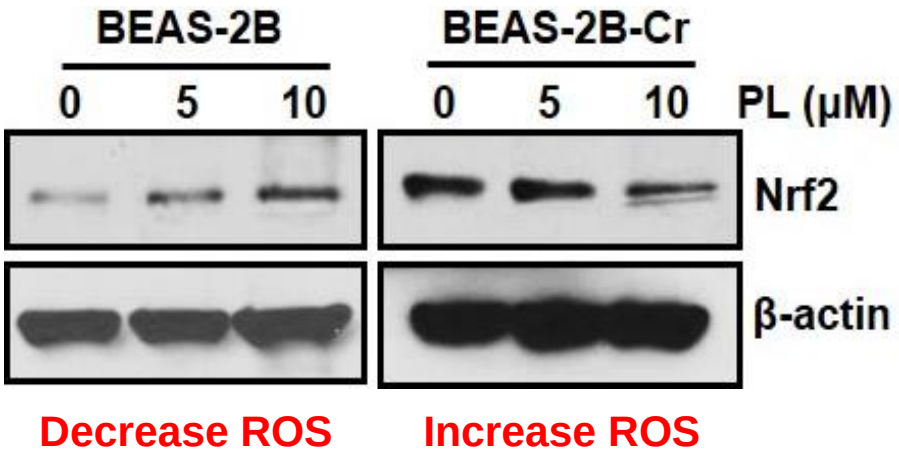
Cell viability by clonogenic assay: Beas-2B cells were treated with Luteolin (0, 20 μM) for 48 h, reseeded and cultured in drug free medium for an additional 2 weeks and stained with crystal violet.

Luteolin reduces Cr (VI) induced cytotoxicity by cologenic assay

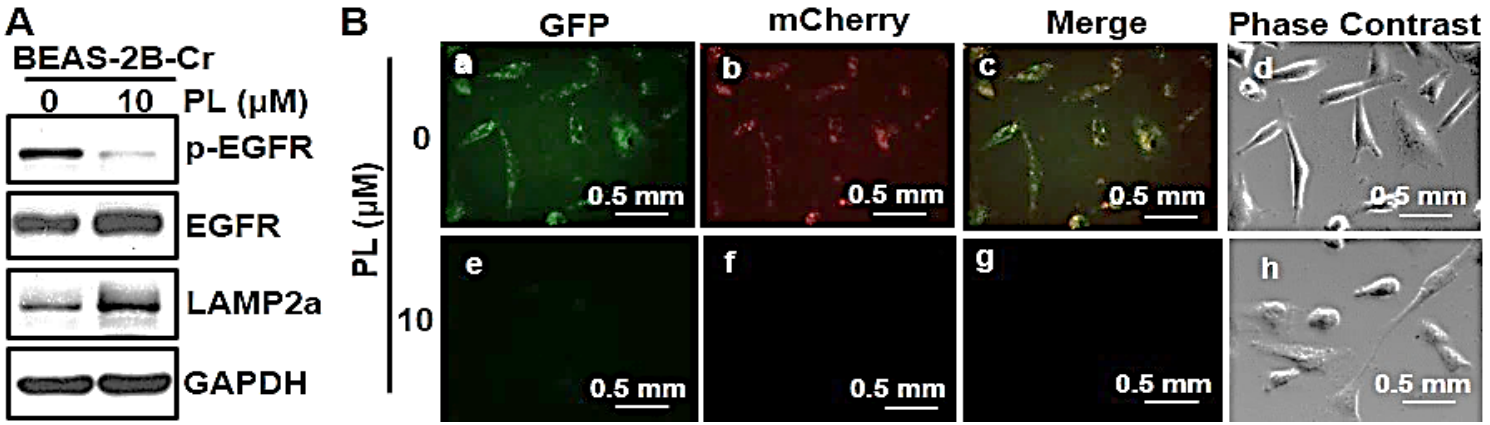




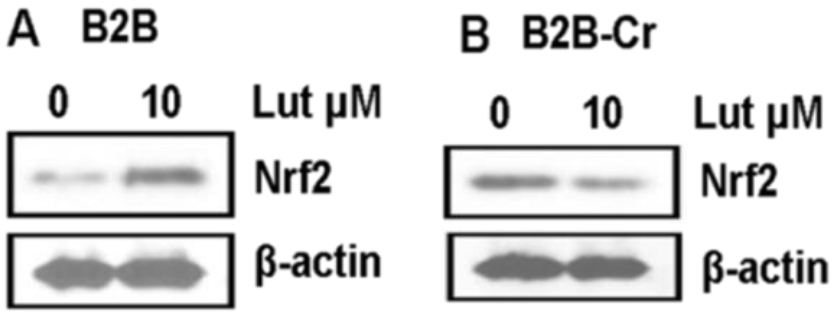
Dual roles of piperlongumine on Nrf2 in normal and Cr(VI)-transformed cells



Piperlongumine inhibits EGFR and autophagy initiation in Cr(VI)-transformed cells.

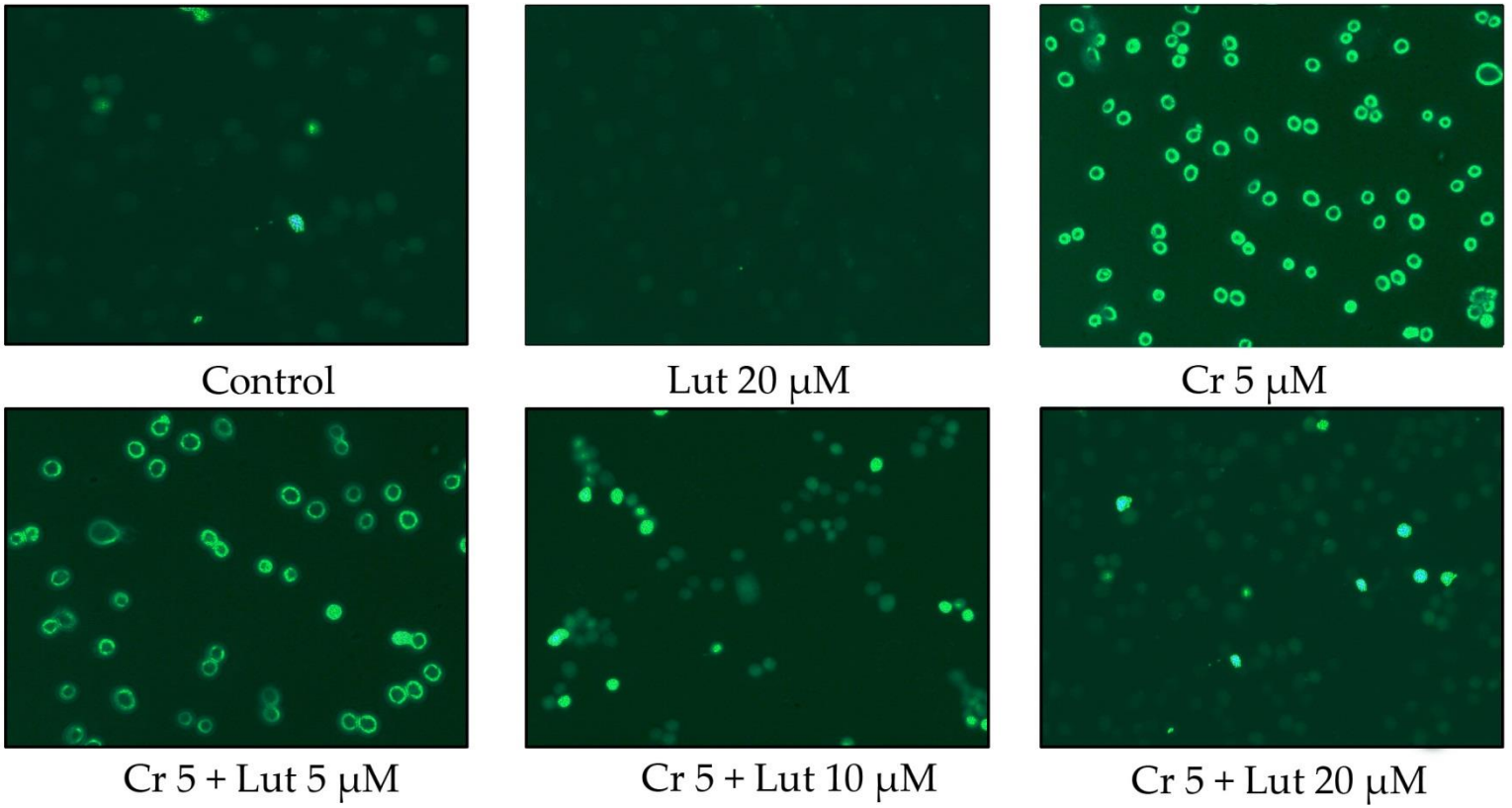


Luteolin increases inducible Nrf2 expression in BEAS-2B cells but decreases constitutive Nrf2 expression in Cr(VI)-transformed BEAS-2B cells



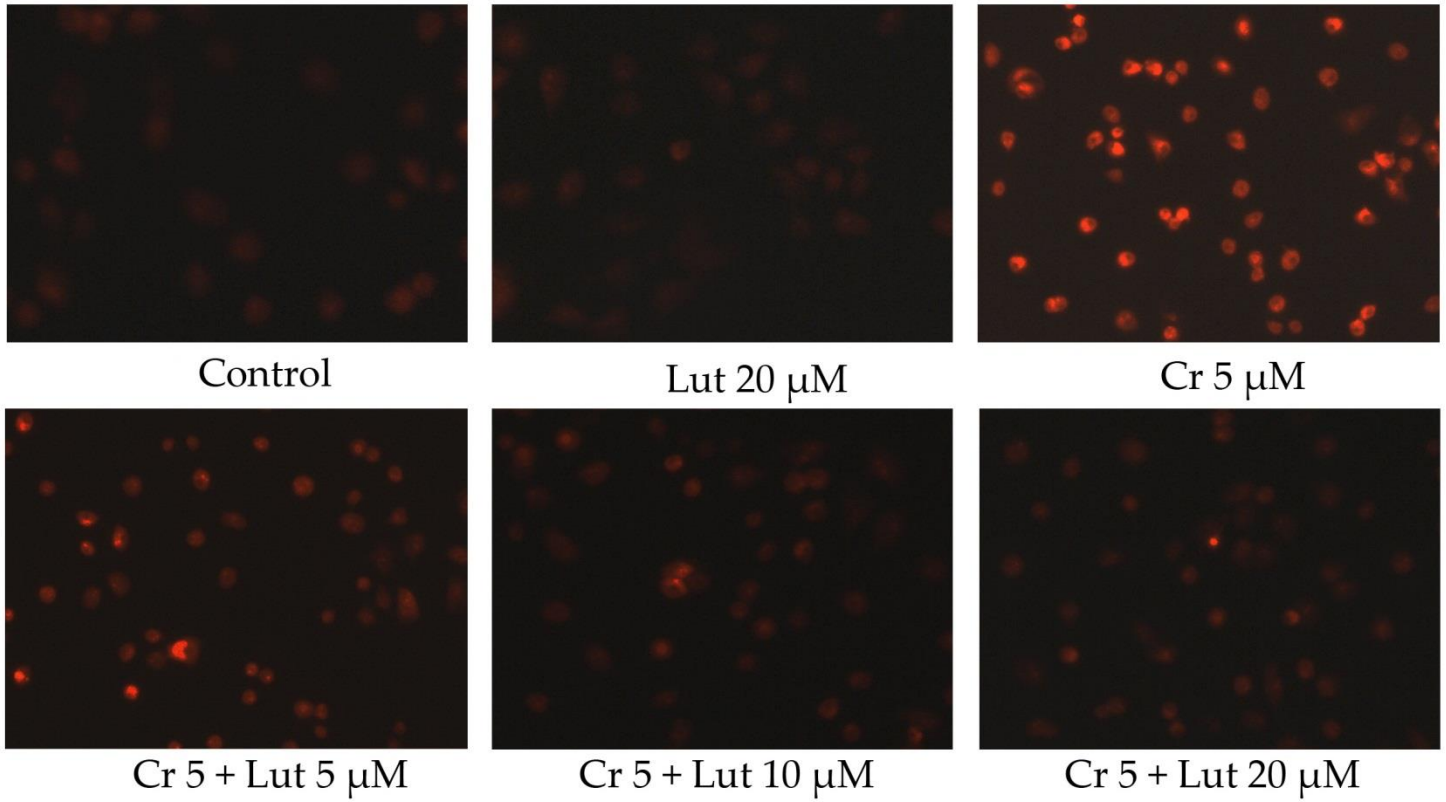
Luteolin increased activation of Nrf2 in normal cells via inhibition of Nrf2 and Keap1 (inhibitor of Nrf2) interaction

Luteolin inhibits Cr(VI)-induced ROS generation in Beas-2B cells



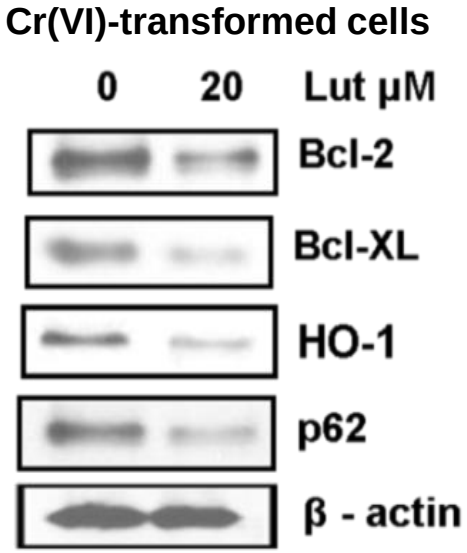
Beas-2B cells were exposed to Cr(VI) (0 or 5 μM)) with or without Luteolin (0, 5, 10, 20 μM) for 6 h and then were labeled with DCFDA (10 μM). Flourescence micrographs were taken.

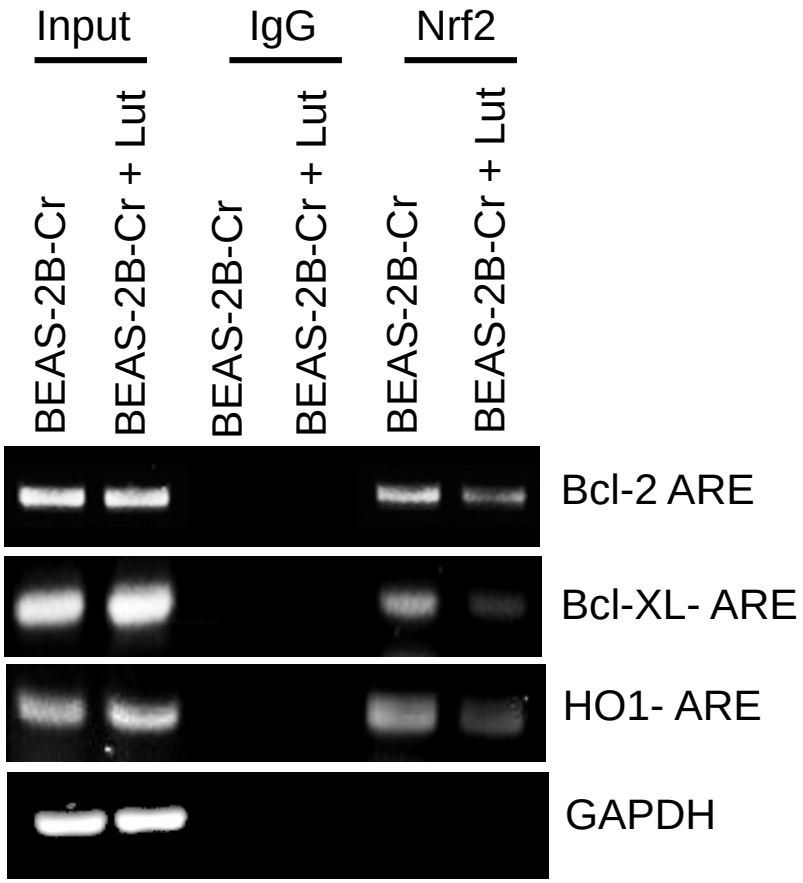
Luteolin inhibits Cr(VI)-induced ROS generation in Beas-2B cells

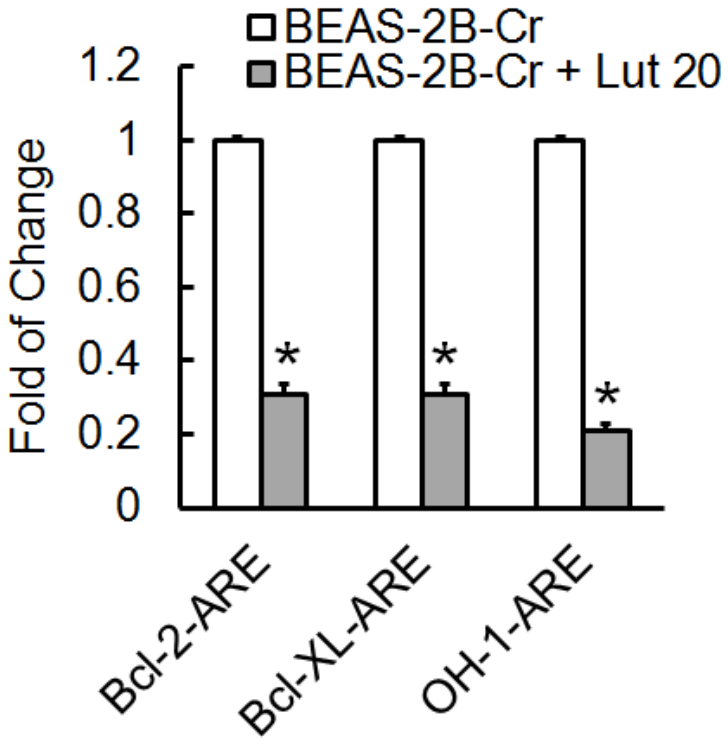


Beas-2B cells were exposed to Cr(VI) (0 or 5 μM)) with or without Luteolin (0, 5, 10, 20 μM) for 6 h and then were labeled with DHE (10 μM). Flourescence micrographs were taken.

Luteolin inhibits constitutive expressions of Bcl-2, Bcl-XL, HO-1, and p62.



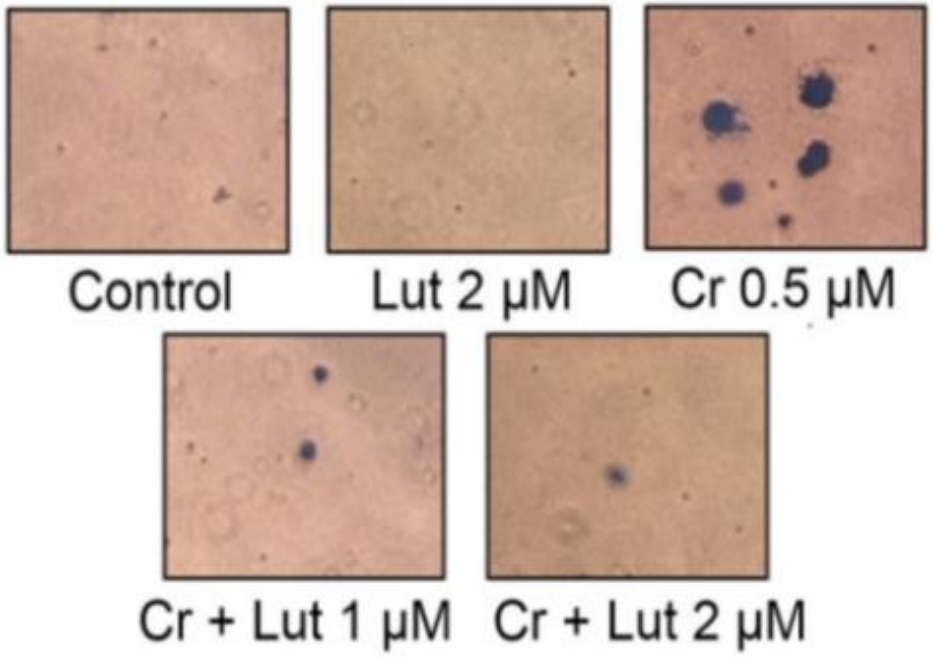




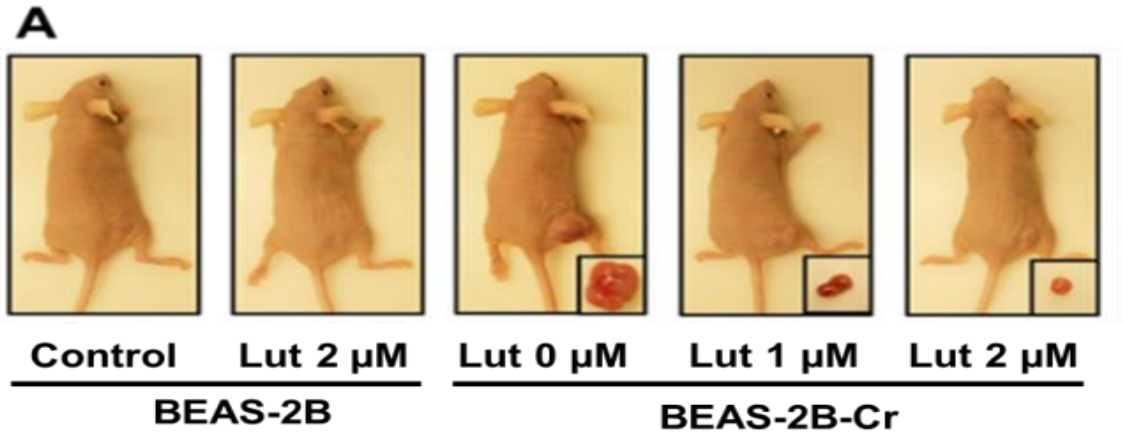
Binding of Nrf2 to ARE of Bcl-2, Bcl-XL and HO-1

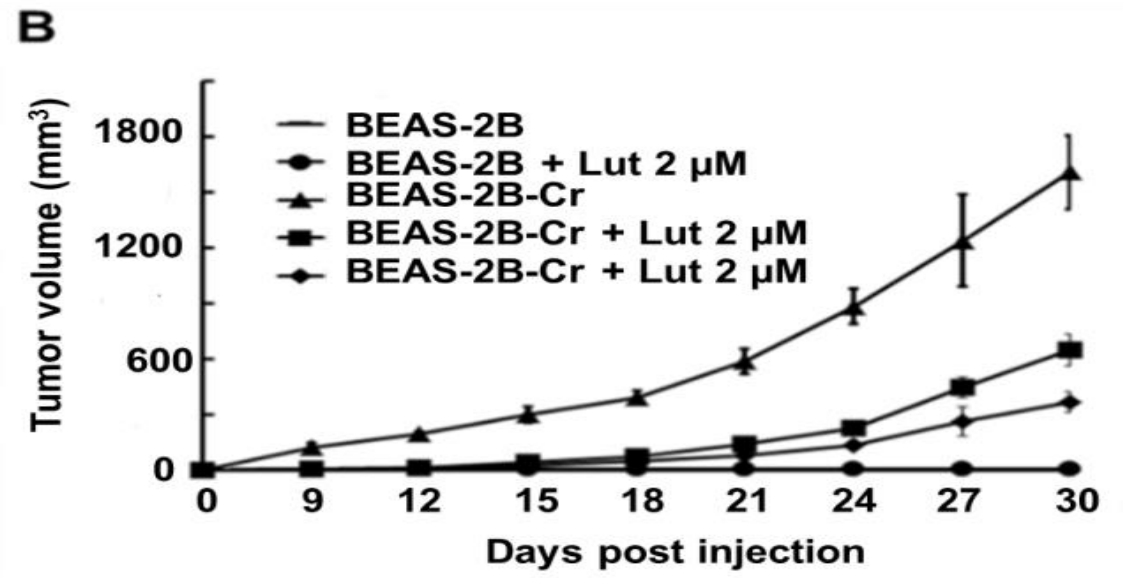
chromatin immunoprecipitation (ChIP) assay

Inhibition of luteolin on Cr(VI)-induced cell transformation

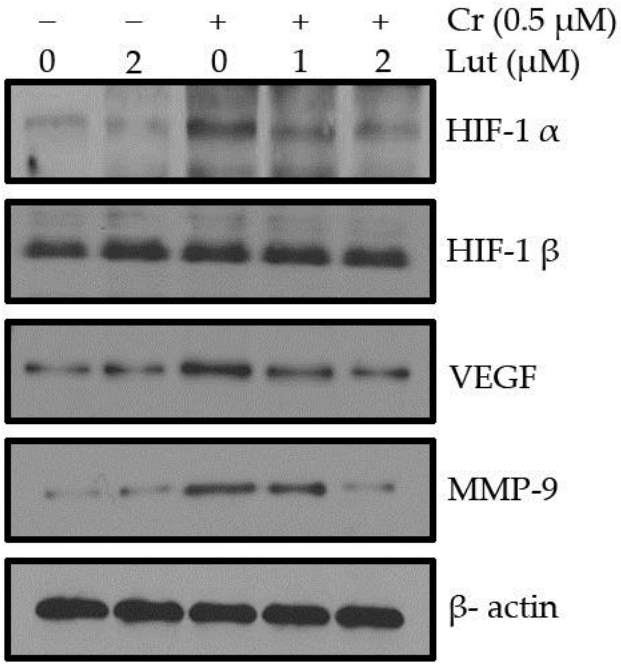


Luteolin protects tumorigenesis of Cr(VI)- transformed BEAS-2B cells

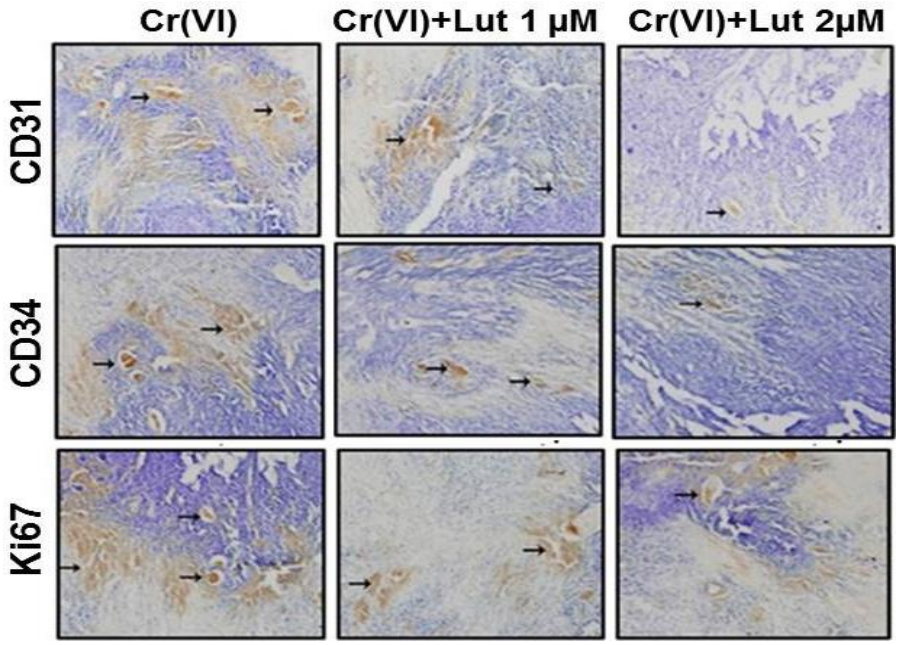




Luteolin inhibits angiogenesis induced by chronic Cr(VI) exposure



Luteolin inhibits Cr(VI)-transformed cell-induced angiogenesis and proliferation in vivo.



1. CD31 and CD34, well known markers of blood vessel endothelial cells, represent microvessle formation.

2. Ki67 is a marker of tumor cell proliferation.

Overall conclusions

1. In normal cells, Cr(VI) generates ROS, which causes cell malignant transformation.

2. In transformed cells:
EGFR is activated, which initiates autophagy, causes autophagy deficiency, and generates p62, leading to constitutive Nrf2 activation.

Consequence
(a) Increase in antioxidant proteins
(b) Decrease in ROS
(c) Increase in anti-apoptosis proteins, Bcl-2 and Bcl-XL, and inflammatory proteins
(d) Apoptosis resistance
(e) Increase HIF-1α and cause angiogenesis

3. Prevention strategy by natural compounds: Increase inducible Nrf2 in normal cells (first stage) (decrease ROS) and decrease constitutive Nrf2 (increase ROS) in transformed cells (second stage) through decrease of EGFR by natural compounds

Results: Reverse (a)–(e) by natural compounds.

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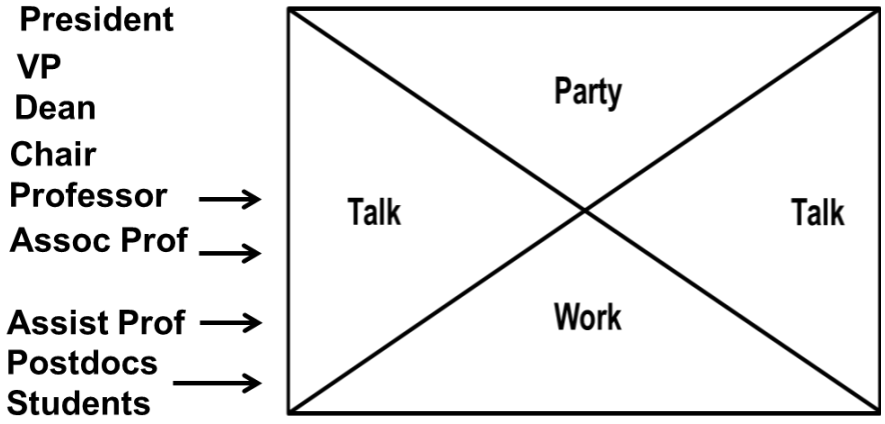


This is the mechanism
of Cr(VI) carcinogenesis