

# The possible role of panitumumab on redox status of colon cancer cell lines DLD-1 and HT-29

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- On September 27, 2006, the FDA granted approval to panitumumab (Vectibix™, Amgen, Inc., Thousand Oaks, CA) for the treatment of patients with EGFR-expressing, metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine-, oxaliplatin-, and irrinotecan- containing chemotherapy regimens (Giusti *et al.*, 2007).

- Panitumumab is a human IgG<sub>2</sub> monoclonal antibody that binds to EGFR and inhibits its phosphorylation and therefore its signaling pathway activation (Giusti *et al.*, 2007).

- Colorectal cancer is the fourth most prevalent carcinoma in Western society and the second cause of cancer death (Rainis *et al.*, 2007).
- Inherited and acquired genetic alterations are the ultimate underlying mechanisms of colorectal carcinogenesis (Volgenstein *et al.*, 1998).
- However, genetic environmental interactions appear to be essential for the development of most cancers (Volgenstein *et al.*, 1998).

- The gastrointestinal tract, especially the colon, is constantly exposed to reactive oxygen species (ROS) originating from endogenous and exogenous sources (Blau *et al.*, 1999).
- An excess of intracellular ROS results in an oxidative environment which modulates gene expression or damages cellular responses (Babbs *et al.*, 1990).

- UV light
- X rays
- Gamma rays

atmosphere

**ROS and RNS**

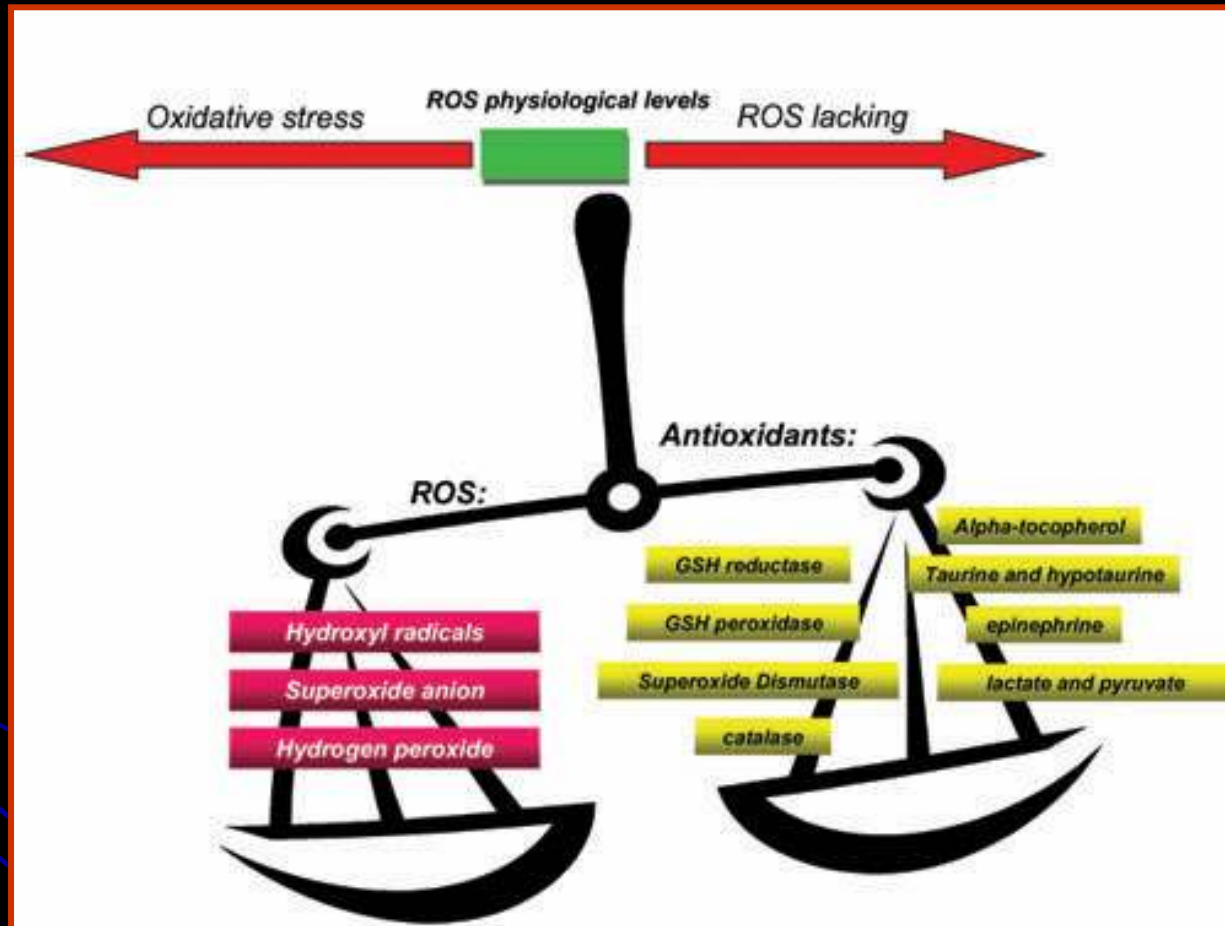
Products of  
meta-catalyzed  
reactions

Neutrophils and  
macrophages during  
inflammation

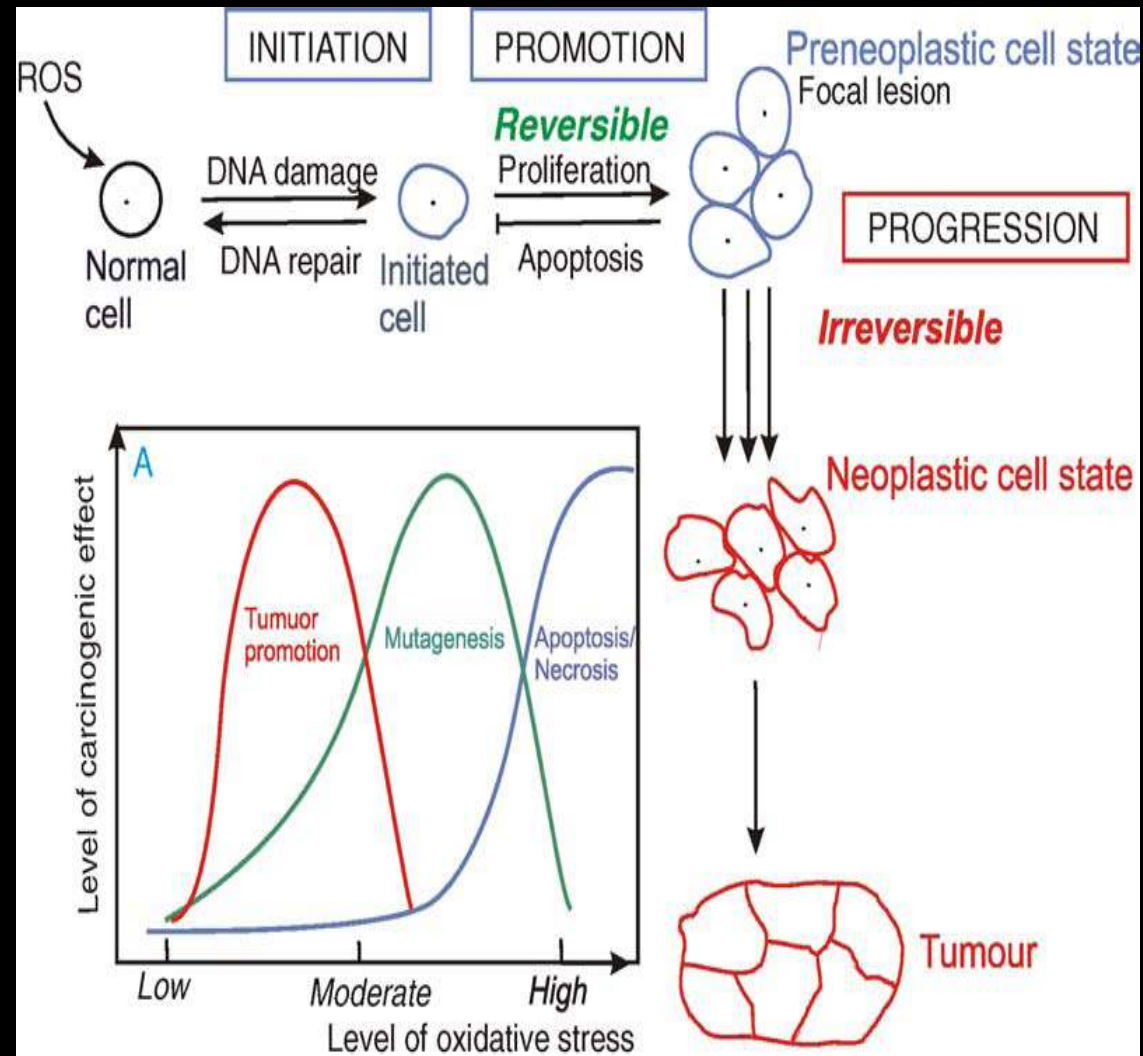
By-products of  
mitochondria-  
catalyzed electron  
transport reactions,  
other mechanisms

Valko *et al.*, 2006

# Dual role of ROS/RNS in biological systems



# The level of carcinogenic effect vs level of ROS at various stages of carcinogenic process



Valko et al., 2006

# Cell defense system

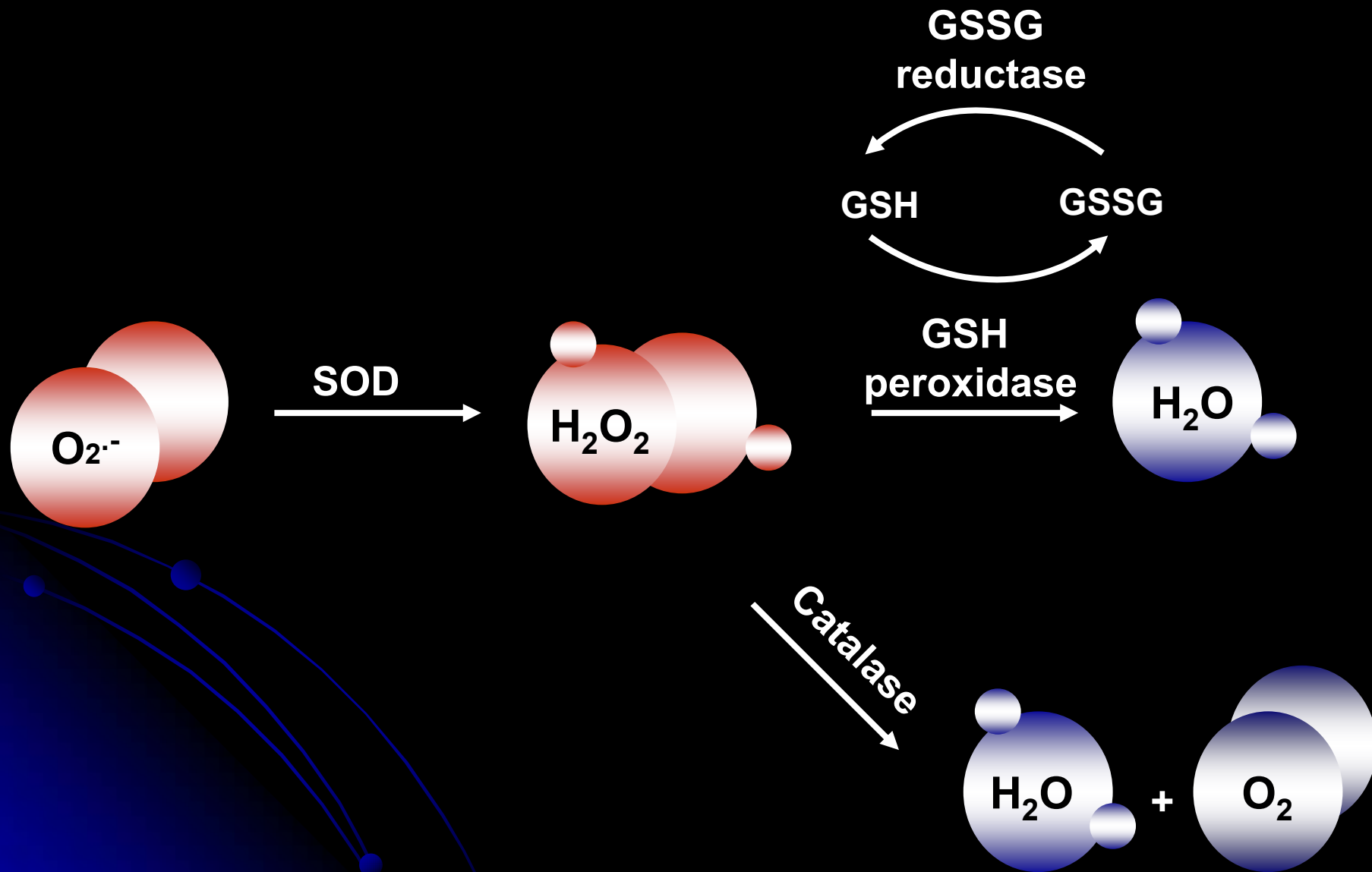
## Enzymatic antioxidants

- Superoxide dismutase (SOD)
- Catalase
- Glutathione peroxidase

## Non enzymatic antioxidants

- Vitamin C, Vitamin E
- Carotenoids
- Thiol antioxidants (GSH, Thx, Lipoic acid)
- Natural flavonoids

# Enzymatic antioxidants

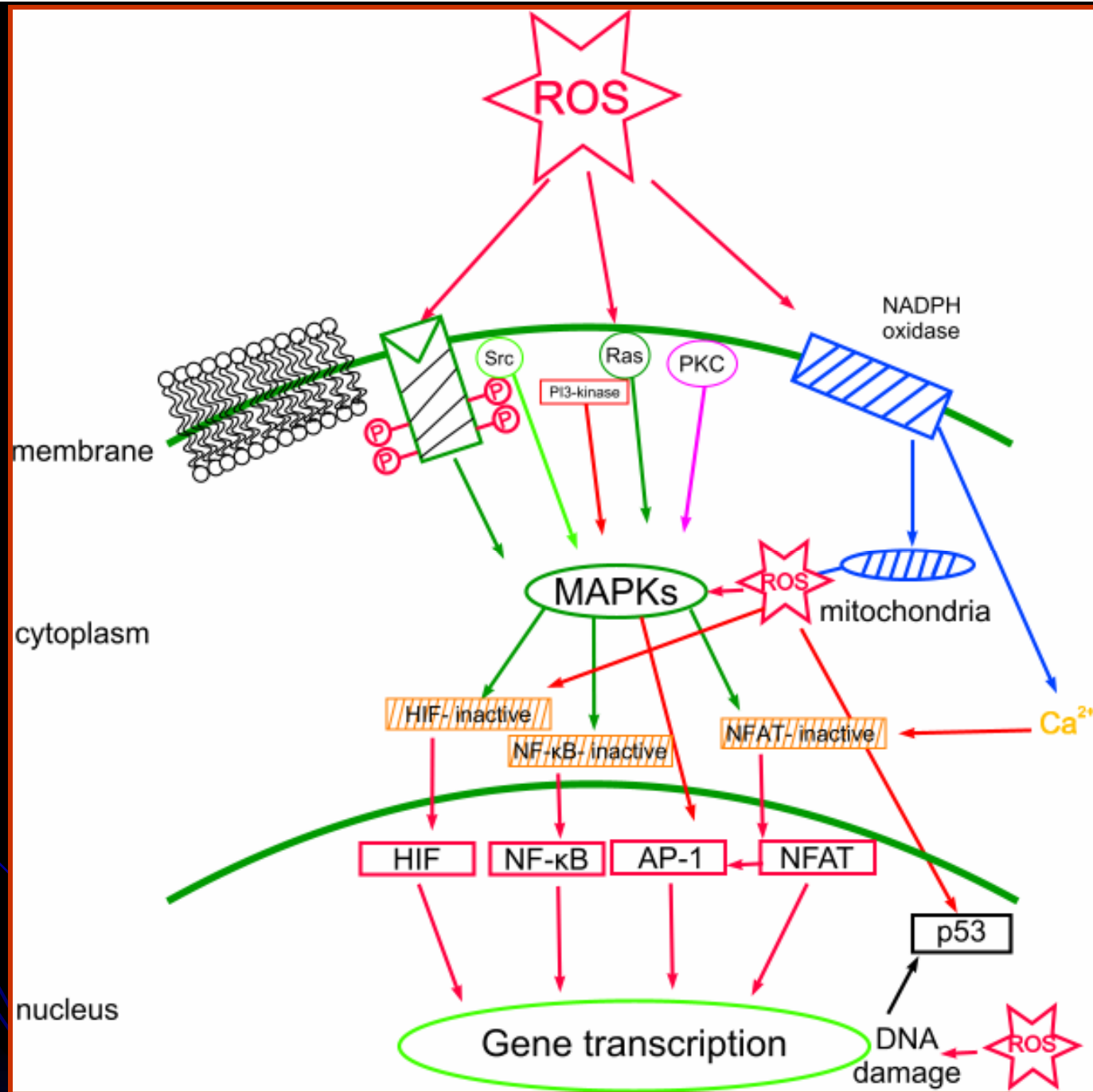


- Previous studies have shown that overexpression of Mn-SOD leads to tumor growth arrest although there are data that correlate high Mn-SOD with poor prognosis, advanced stages of progression and an invasive and metastatic phenotype (Behrend *et al.*, 2003).
- An overexpression of Mn-SOD has been detected in cancers of gastrointestinal tract (Behrend *et al.*, 2003).
- There is a link between decreased capacity of tumors to detoxify  $H_2O_2$  and a decreased level of catalase (Valko *et al.*, 2006).
- There are data that revealed an increase in activity of glutathione peroxidase and glutathione reductase in patients with colorectal cancer (Skrzydowska *et al.*, 2005).

## Non enzymatic antioxidants

- The main protective role of GSH against oxidative stress is the regeneration of the most important antioxidants: vitamin C and E to their active forms. This capacity of GSH is linked with the redox state of the GSSG/2GSH. This in turn has a high impact on the overall redox environment of the cell (Valko *et al.*, 2006).
- It has been found that the levels of Vitamin C, E and GSH are decreased with the progression of colorectal cancer (Skrzydłewska *et al.*, 2005).

# ROS induced signaling pathways



Valko et al., 2006

## ROS and EGFR signaling

There are data that (Liu *et al.*, 2006):

- $\text{H}_2\text{O}_2$  is required for EGF-induced PI3K/AKT activation
- $\text{H}_2\text{O}_2$  mediated EGF-induced VEGF expression

Very few data exist for colorectal cancer (Rainis *et al.*, 2007)

## **Aim:**

**The aim of this study is to investigate the implication of panitumumab on the redox status of two human colon cancer cell lines DLD-1 and HT-29.**

## Characteristics of DLD-1 and HT-29 cells

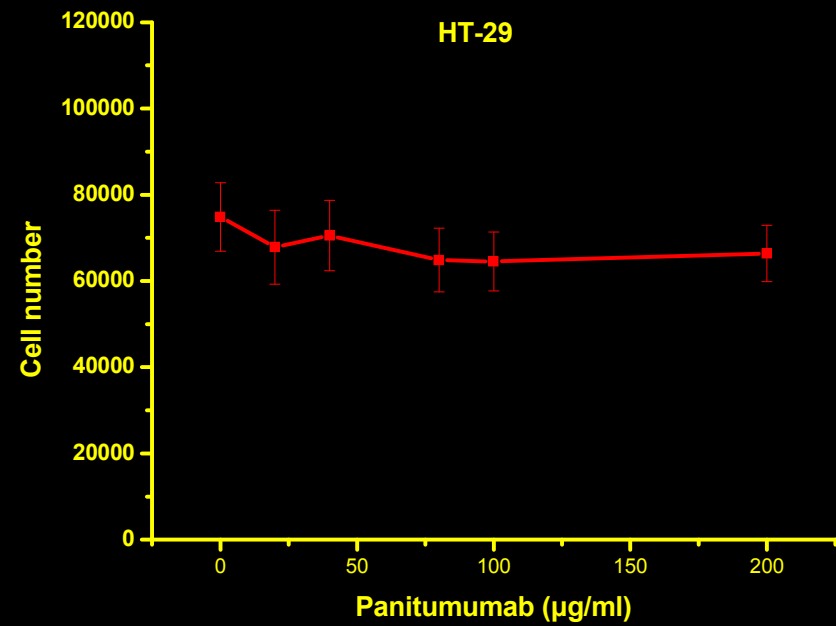
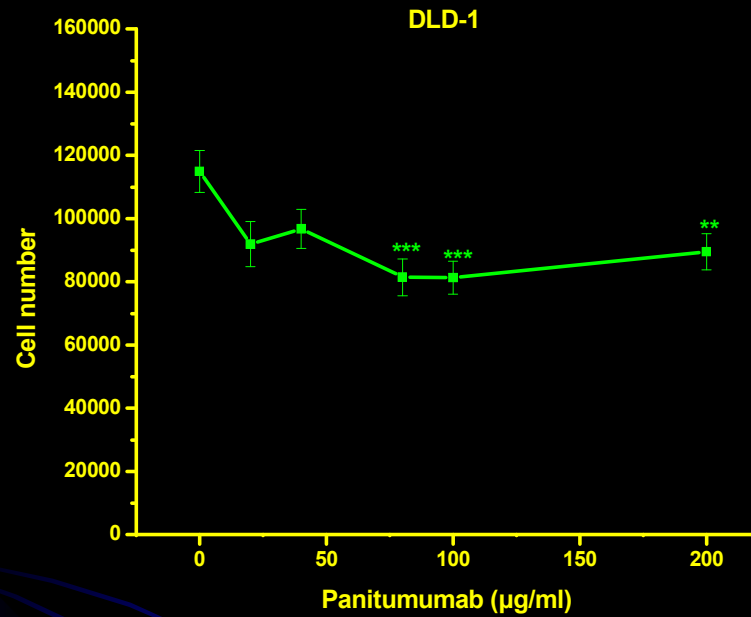
```
graph TD; A[Characteristics of DLD-1 and HT-29 cells] --> B[DLD-1  
High levels of active Kras  
Positive for EGFR]; A --> C[HT-29  
Low levels of active Kras  
Positive for EGFR];
```

DLD-1  
High levels of active Kras  
Positive for EGFR

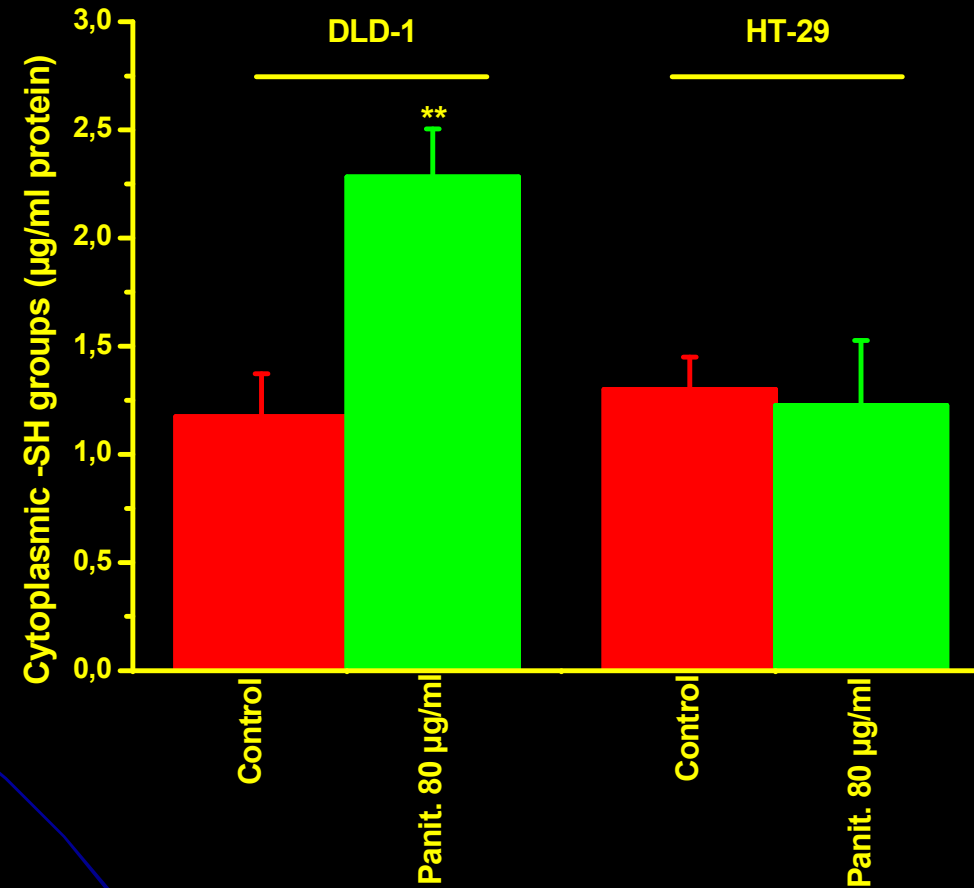
HT-29  
Low levels of active Kras  
Positive for EGFR

- Cells were treated with several concentrations of panitumumab: 20, 40, 80, 100 and 200  $\mu\text{g/ml}$
- IgG antibody was used as control

# The effect of panitumumab in cell proliferation



# The effect of panitumumab in -SH groups levels



## The effect of panitumumab on cell death

- Panitumumab didn't affect cell apoptosis or necrosis or cell cycle in DLD-1 cells.
- GSH protects cells against apoptosis — the protective role originates from multifactorial mechanisms that involve detoxification and modulation of cellular redox state and the subsequent redox-sensitive cell-signalling pathways and interaction with pro- and anti-apoptotic signals.

## How does panitumumab kill cells?

- Panitumumab maybe kill cells through autophagy.
- Autophagic cell death has traditionally been classified as non apoptotic death that separate from necrosis. Briefly, a double membrane vesicle forms in the cytosol that encapsulates whole organelles and bulk cytoplasm. This autophagosome then fuses with the lysosome where the contents are degraded and recycled (Edinger *et al.*, 2004).
- A recent report (Cancer cell, Weihua *et al.*, 2008) refers that inhibition of EGFR leads to autophagic cell death in prostate, breast, skin and colon cancer cell lines.

## Conclusions

- Results from previous studies show that colorectal carcinogenesis is associated with serious oxidative stress and that advancement of oxidative-antioxidative disorders is followed by progression of colorectal cancer.
- Our preliminary data showed that panitumumab affected proliferation in cells with high activity of Kras.
- Panitumumab increased cytoplasmic –SH groups levels altering the redox status of cells with high activity of Kras.

Panitumumab may exert its antitumor activity through EGFR inhibition although it seems that the redox status of cells is very important. Any change in the oxidative/reductive state in cell may contribute to the antitumor effect of panitumumab.

To be continued...