The HIF pathway and reactive oxygen species – regulation of the anti-tumor effect of gas plasma?

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Introduction

Gas plasma represents a new form of therapy for cancerous diseases, the molecular effects of which are not yet fully understood. Gas plasma reacts with ambient oxygen to produce reactive oxygen species (ROS) and is likely to affect the hypoxia-inducible factor (HIF) pathway by the induction of local hypoxia and/or ROS, affecting cellular oxygen sensors. Furthermore, one of the oxygen sensors, factor inhibiting HIF (FIH) can additionally sense H_2O_2. The oxygen sensors regulate the transcription factor HIF, which in turn regulates cell apoptosis, resistance against oxidative stress and ROS-production by mitochondria. **The aim of this work is to investigate the interaction between ROS and cellular oxygen sensors in more detail, as this may effect and shape the tumor cell response to gas plasma treatment.**

Methods

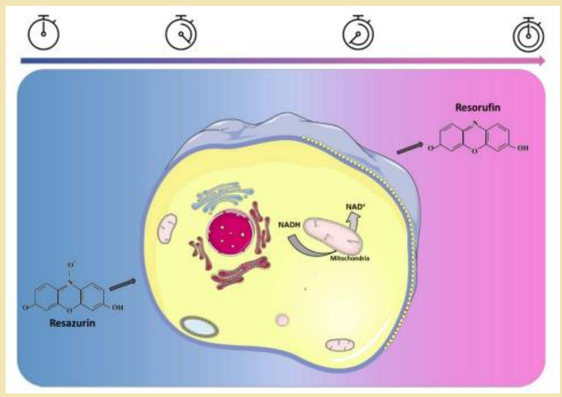

Fig.1: Reduction of resazurin into resorufin in viable cells(1).

Cell lines:

Cancerous

A375 (malignant melanoma)
A549 (lung adenocarcinoma)
HeLa (cervical carcinoma)
HT-29 (colon carcinoma)

Non-cancerous

HaCaT (keratinocytes)
HEK293 (human embryonic kidney cells)

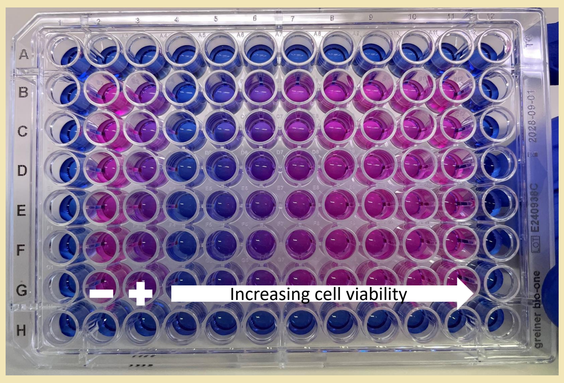

Fig.2: Example of a 96-well plate with A375 treated with hydrogenperoxid:
neg. contr.(-); pos. contr. (+);
dilution series (→)

Resazurin assay:

The cells were seeded into a 96-well plate and incubated for 24 h. Subsequently, the cells were treated with different concentrations of hypochlorous acid (NaClO), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) for additional 24h. These substances are the most commonly produced ROS while tissue is treated with gas plasma and crucial for the induced anti-tumor effects, leading to apoptosis and cell death.

To measure the vitality of the cells, an fluorescence assay is performed using resazurin.

Metabolically active cells reduce the blue colored resazurin into the pink fluorescent resorufin, demonstrating viability. The fluorescence is measured 4 hours after addition of resazurin with a 540 nm excitation/ 590 nm emission filter set.

The measured fluorescence is proportional to the number of metabolically active cells in each well.

Following at least three independent measurements, EC_{35}-values were determined.

Results

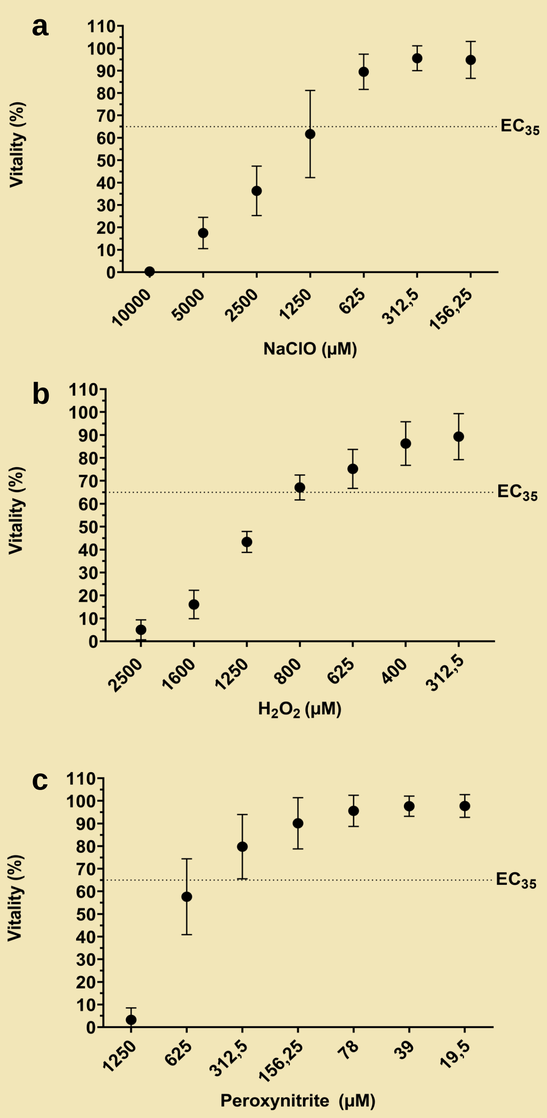

Fig. 3: Cell death induced in A375 cells with the following different oxidative agents.
EC_{35}-values determined :
(a) treated with NaClO (n=4),
EC_{35}=1151 µM;
(b) treated with H_2O_2 (n=3),
EC_{35}= 832 µM;
(c) treated with $ONOO^-$ (n=3),
EC_{35}=496 µM

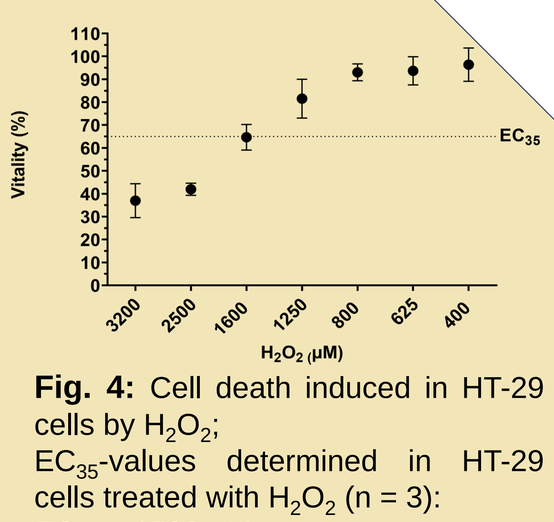

Fig. 4: Cell death induced in HT-29 cells by H_2O_2;
EC_{35}-values determined in HT-29 cells treated with H_2O_2 (n = 3):
EC_{35} = 1592 µM.

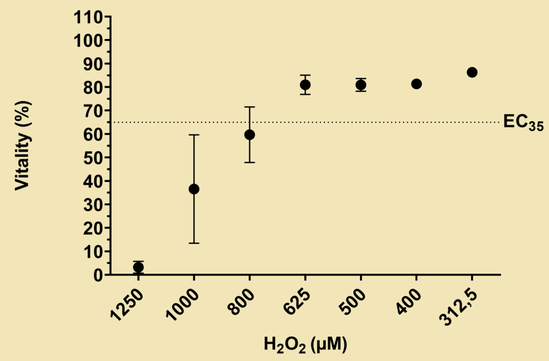

Fig. 5: Cell death induced in HEK293 cells by H_2O_2;
EC_{35}-values determined in HEK293 cells: treated with H_2O_2 (n = 4), EC_{35}= 752 µM

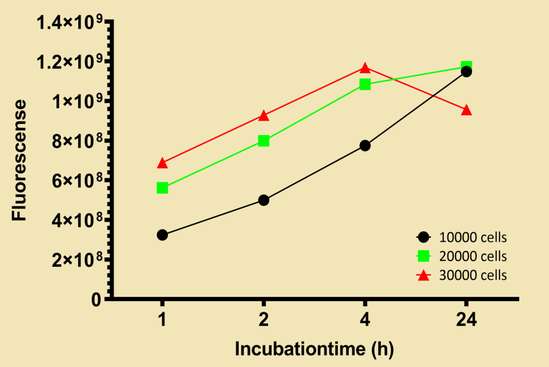

Fig. 6: Fluorescence of different cell numbers per well with HaCaT cells measured 1, 2, 4 & 24 hours after resazurin was added.

Roadmap

Determination of the EC_{35}-values for H_2O_2, NaClO and $ONOO^-$ in (non-)cancerous cell lines

Investigation of the functional interaction between ROS and cellular oxygen sensors via PHD/FIH-inhibitors

Investigation of the interaction between gas plasma and cellular oxygen sensors

Summary

- The used resazurin assay is a sensitive, reliable and fast method to measure the vitality of cultured cells
- Different (non-)cancerous cell lines display various sensitivities for ROS
- Single (non-)cancerous cell lines vary in their sensitivity to different ROS-inducing agents

(1)Petiti et al., Biosensors 2024;14: 156.