

# The Effect Of Hypoxia On Prolidase – Dependent Activation of Apoptosis in Breast Cancer Cells

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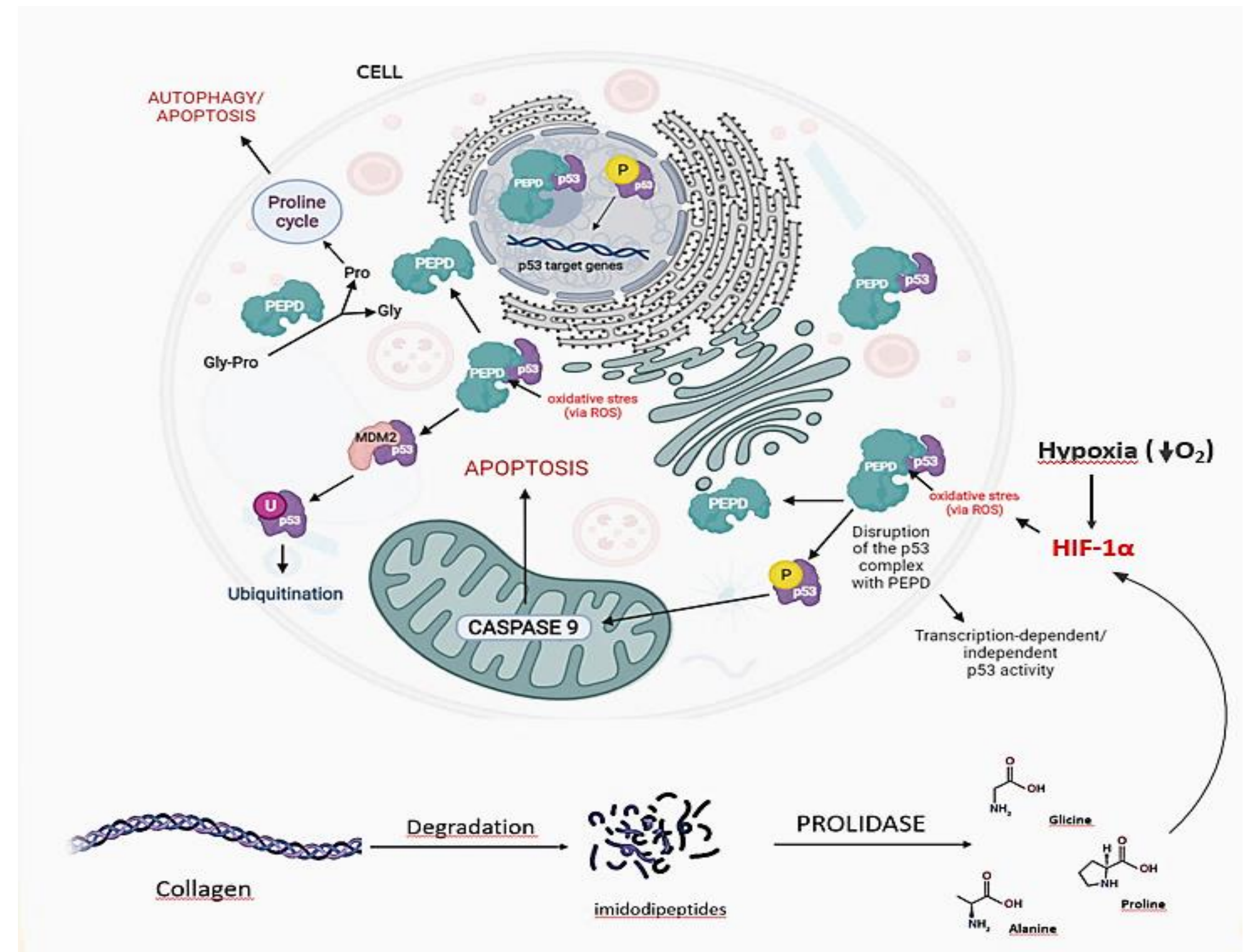
## ABSTRACT

Hypoxia is a condition that characterizes the microenvironment created by neoplastic cells that are in new development and rapidly growing. It is accompanied by a number of cell metabolic process abnormalities, and a consequence of hypoxia is the stabilization of the hypoxia-induced factor HIF1 $\alpha$ . The state of hypoxia is also a factor inducing the formation of reactive oxygen species, predispose to the activation of apoptosis. The process of intrinsic apoptosis depends on the status of the p53 protein. One of the regulators of the expression of this protein is Prolidase (PEPD). This enzyme has the ability to bind and inactivate the function of p53, thereby blocking the induction of apoptosis by, for example, chemotherapeutics. One of the molecular reasons for this phenomenon may be the level of intracellular Prolidase. The aim of this study was to investigate the effect of hypoxia-induced oxidative stress on the activation of p53-dependent apoptosis in breast cancer cells with different levels of PEPD expression.

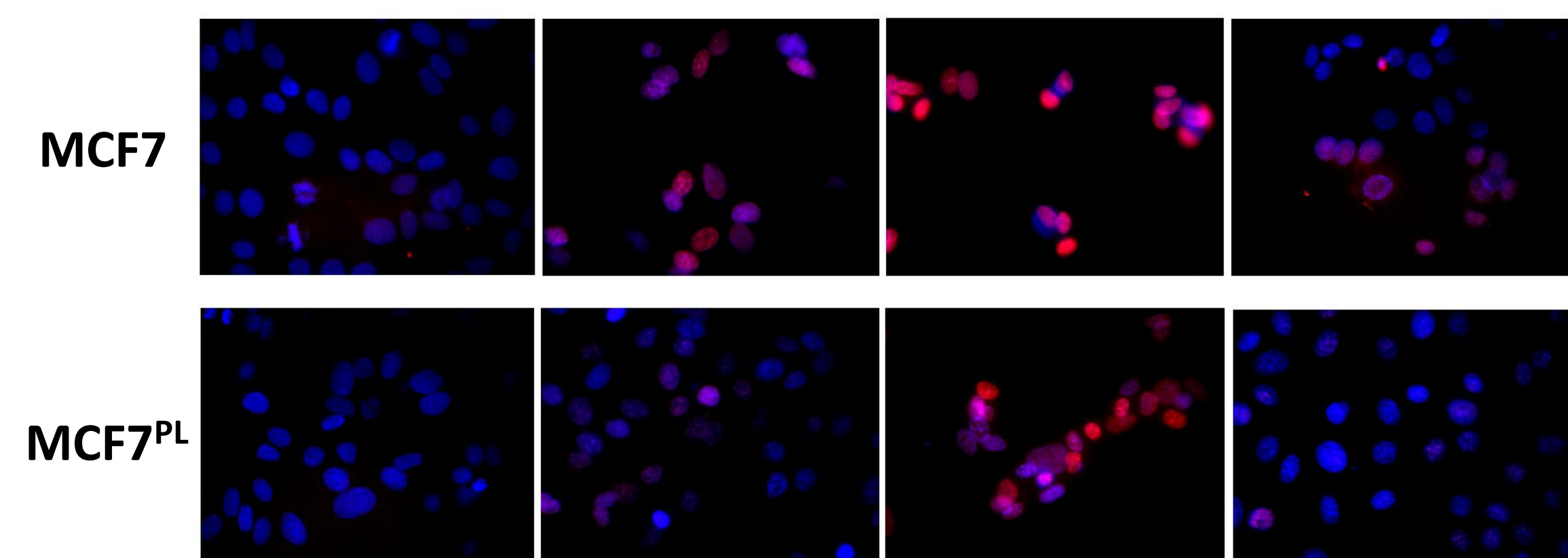
MCF7 (wild type), MCF7 with prolidase overexpression (MCF7<sup>PL</sup>) and a zebrafish model were used. Doxorubicin (a direct p53 activator) was used to induce apoptosis. Hypoxia was used to induce oxidative stress. Cell apoptosis, was measured using Nucleo-Counter NC-3000, DNA biosynthesis by radiometric method. Prolidase activity by a colorimetric method. Expression and translocation of selected proteins were analyzed by fluorescence microscopy and WB.

Results: Doxorubicin under both normoxia and hypoxia conditions decreased cell survival and DNA biosynthesis, increasing apoptosis, but its effect was markedly more potent in cells with low level of Prolidase expression (MCF7). The hypoxic condition and the ROS generated enhanced apoptosis, probably as a result of dissociation of the PEPD-p53 complex and an increase in the pool of free p53 fraction, as indicated by the changes observed in cells with PEPD overexpression (MCF7<sup>PL</sup>). Doxorubicin induced apoptosis in a dose-dependent manner, while this effect was observed at a much lower level in MCF7<sup>PL</sup> cells. Prolidase thus has a pro-survival role, protecting cells from p53-dependent activation of apoptosis. Doxorubicin may be a candidate for treating cancers with impaired response to therapy whose molecular basis is PEPD overexpression.

Conclusions: Overexpression of Prolidase in MCF7 cells (MCF7<sup>PL</sup>) counteracts p53-dependent apoptosis induced by doxorubicin. On the other hand, a state of hypoxia through induction of oxidative stress enhanced apoptosis, especially in MCF7<sup>PL</sup> cells. Thus, combination therapy: a drug that initiates p53-dependent apoptosis with an inducer of oxidative stress may be a potential therapy for tumors with prolidase overexpression.

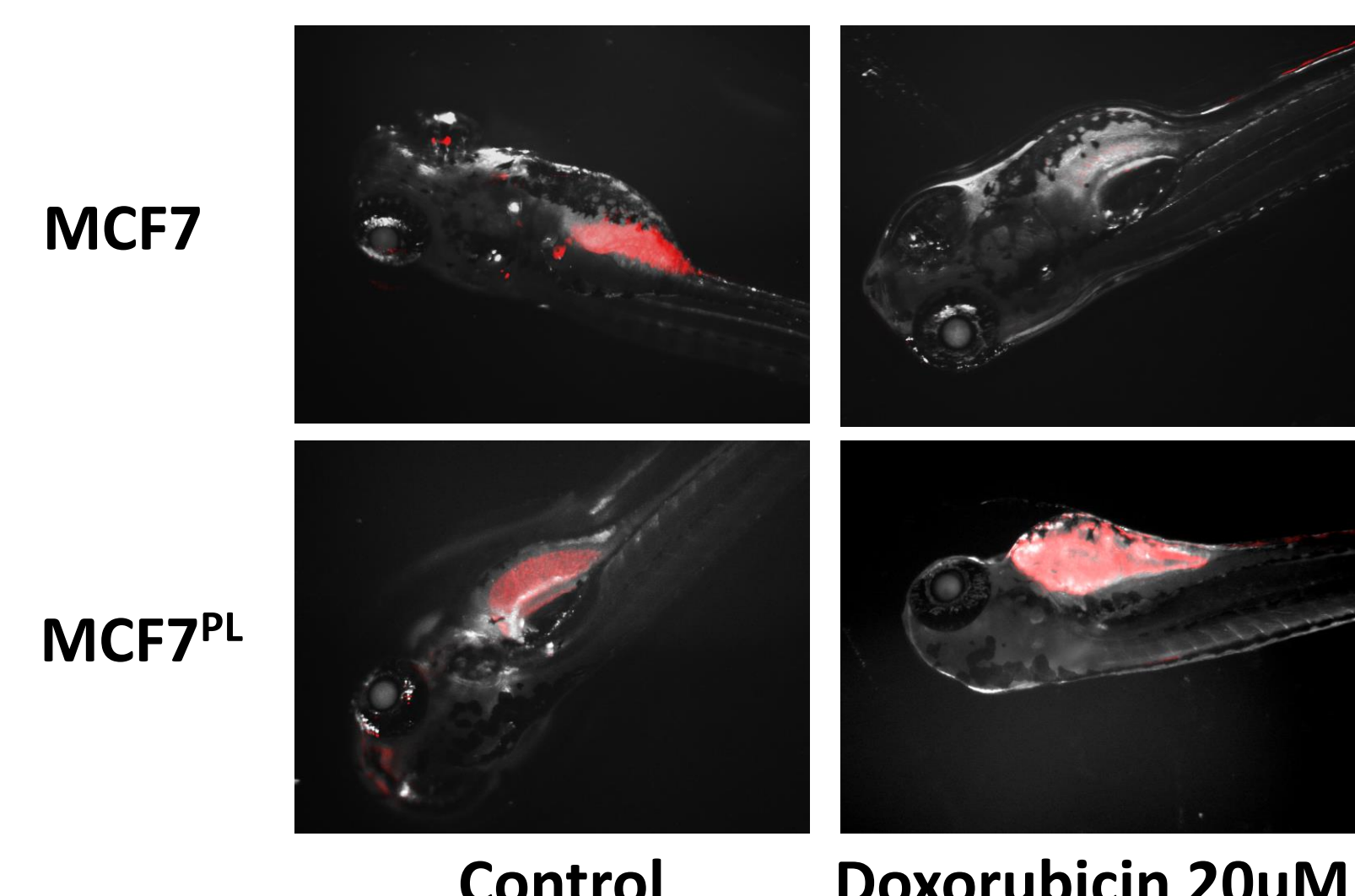


Confocal microscopy bio-imaging of p53 protein in MCF7 and MCF7<sup>PL</sup> cells treated with treated for 24 hours with Doxorubicin, Hypoxia and Vitamin C.



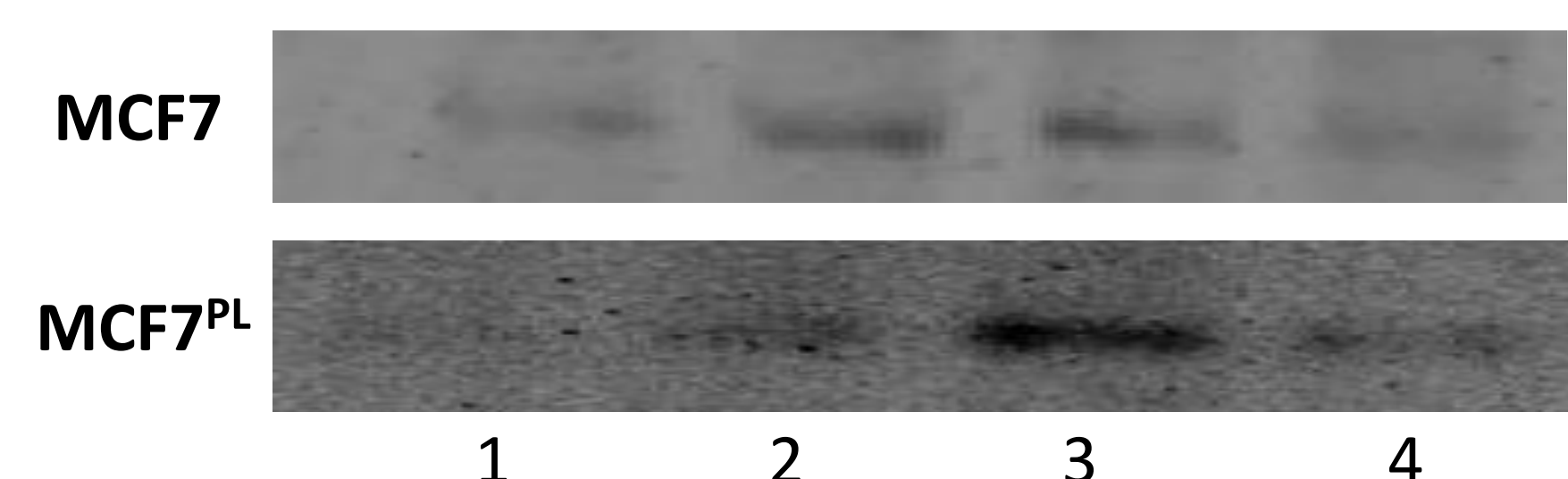
1. Control 2. Doxorubicin 20uM 3. Doxorubicin 20uM + Hypoxia 4. Doxorubicin 20uM + Hypoxia + Vit. C 20uM

Effect of Doxorubicin on MCF7 and MCF7<sup>PL</sup> tumor size using a Zebrafish model.

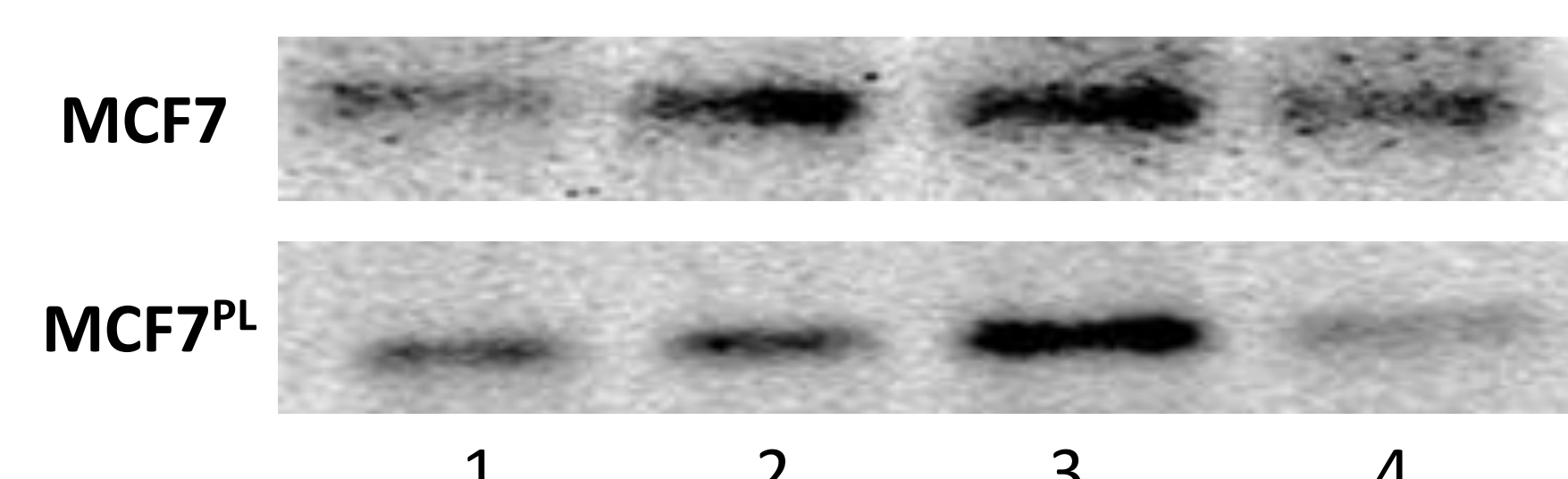


Effect of PEPD overexpression and used substances on expression of active forms of Caspases 7 and 9 in MCF-7 and MCF-7<sup>PL</sup> cells.

## Caspase 7



## Caspase 9



1. Control 2. Doxorubicin 20uM 3. Doxorubicin 20uM + Hypoxia 4. Doxorubicin 20uM + Hypoxia + Vit. C 20uM

## Conclusions

Prolidase plays a pro-survival role, protecting cells from p53-dependent apoptosis activation. The status of hypoxia and the ROS generated increased the apoptosis process, probably due to the dissociation of the PEPD-p53 complex and an increase in the pool of free p53 fraction, as suggested by the changes observed in cells with PEPD overexpression (MCF7<sup>PL</sup>). Doxorubicin may be a candidate for the treatment of cancers with impaired response to therapy, the molecular basis of which is PEPD overexpression. Successful therapy with Doxorubicin has a chance in cancer stages characterized by tumor hypoxia.