NINTH INTERNATIONAL
CONFERENCE ON RADIATION
IN VARIOUS FIELDS OF RESEARCH
June 14-18, 2021 | Hunguest Hotel Sun Resort | Herceg Novi | Montenegro

Hypoxia and antioxidant signaling in human colon carcinoma HCT116 exposed *in vitro* to 64-Cu

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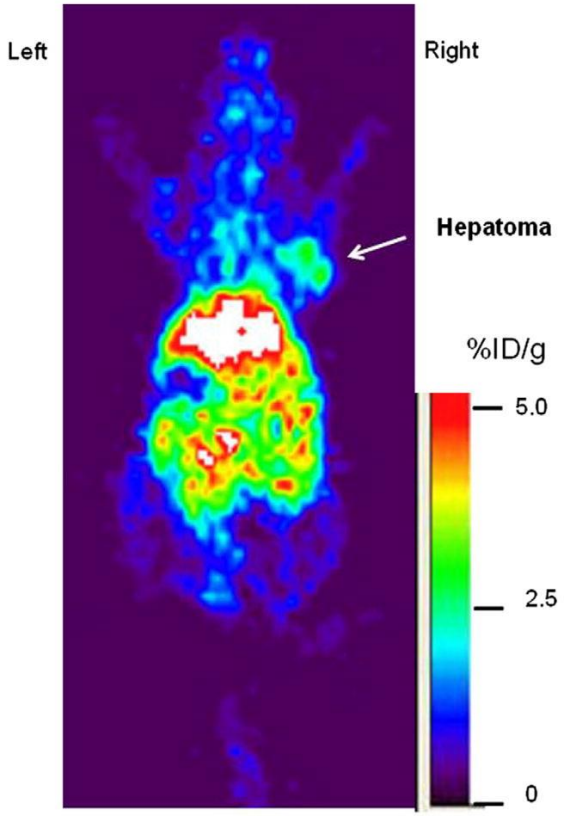
Background. 64-Cu has been lately demonstrated to be a valuable theranostic agent in solid tumors, combining PET-imagistic and therapeutical properties. Nevertheless, there is an urgent need to describe the molecular background underlying its action mechanisms in order to further identify candidate molecular targets aimed at improving the therapeutic outcome as well as the toxicological profile of 64-Cu.



Micro–positron emission tomographic (PET) imaging of human hepatocellular carcinoma (HCC) xenografts in mice with $^{64}CuCl_2$ (2 μCi/g body weight).
The HCC xenograft with increased ^{64}Cu radioactivity was well visualized (*arrow*) on a representative micro-PET image of a tumor-bearing mouse injected with $^{64}CuCl_2$ intravenously.
The color scale indicates tracer concentrations in percentage of injected dose per gram of tissue (%ID/g).

Haiyuan Zhang et al. Positron Emission Tomography of Human Hepatocellular Carcinoma Xenografts in Mice Using Copper (II)-64 Chloride as a Tracer. *Acad Radiol* 2011, 18(12): 1561–1568.

Aim: to identify *in vitro* using a transcriptomic approach the response of normal and tumor cells to 64-Cu, with a particular focus on hypoxia signaling and redox responses.

Method

Cell viability. Human colon carcinoma HCT116 cells and human normal BJ fibroblasts (ATCC) were treated with 20 MBq 64-$CuCl_2$. The number of viable tumor cells in culture was assessed at 24-72 h after radioisotope addition by the MTS reduction test using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) from Promega. Other tumor cell lines were investigated for comparison.

Gene expression. Based on viability data, adherent HT29 colon carcinoma cells and normal BJ fibroblasts were harvested in TRIzol at 24 h for RNA isolation. The gene expression study was performed by qRT-PCR using an array of 84 stress genes (Stress and toxicity pathway finder, Qiagen – see next slide). GAPDH was chosen as housekeeping gene according to RefFinder analysis on 5 housekeeping genes. Genes with a fold of change (FC) > 1.5 were considered for interpretation. FC was calculated based on $2^{-\Delta C_T}$ values of treated *versus* non-treated cells.

Oxidative Stress

FTH1, GCLC, GCLM, GSR, GSTP1, HMOX1, NQO1, PRDX1, SQSTM1, TXN, TXNRD1

Hypoxia Signaling

ADM, ARNT, BNIP3L, CA9, EPO, HMOX1, LDHA, MMP9, SERPINE1 (PAI-1), SLC2A1, VEGFA

Osmotic Stress

AKR1B1, AQP1, AQP2, AQP4, CFTR, EDN1, HSPA4L (OSP94), NFAT5, SLC5A3

Cell Death

Apoptosis*:* CASP1 (ICE), FAS, MCL1, TNFRSF10A (TRAIL-R), TNFRSF10B (DR5), TNFRSF1A (TNFR1).

Autophagy: ATG12, ATG5, ATG7, BECN1, FAS, ULK1

Necrosis*:* FAS, GRB2, PARP1 (ADPRT1), PVR, RIPK1, TNFRSF10A (TRAIL-R), TNFRSF1A (TNFR1), TXNL4B

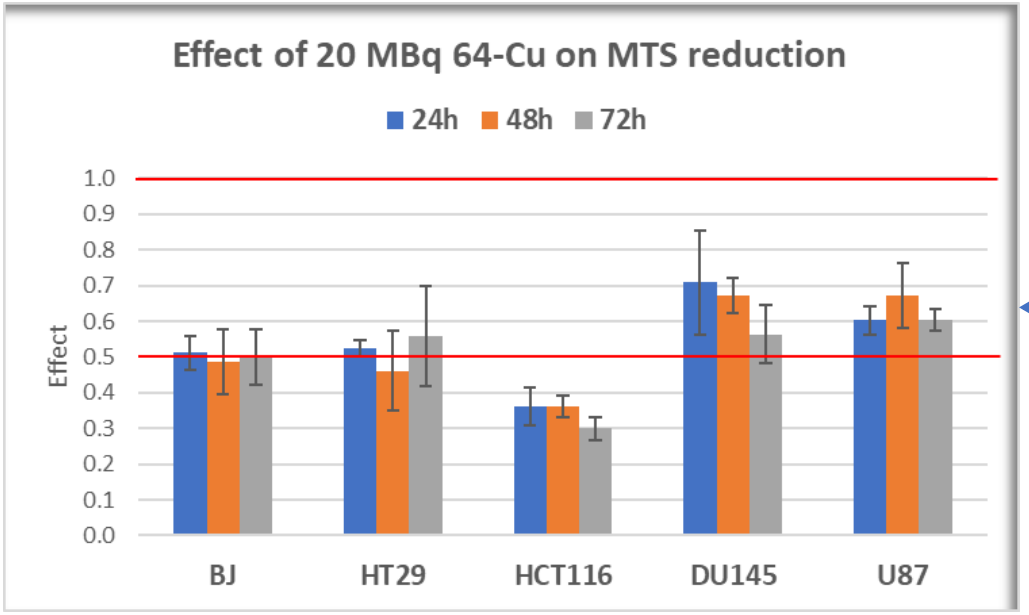
DNA Damage & Repair

Cell Cycle Arrest & Checkpoints: CDKN1A (p21CIP1, WAF1), CHEK1, CHEK2 (RAD53), DDIT3 (GADD153, CHOP), HUS1, MRE11, NBN, RAD17, RAD9A

Other DNA Damage Responses*:* ATM, ATR, DDB2, GADD45A, GADD45G, RAD51, TP53 (p53), XPC

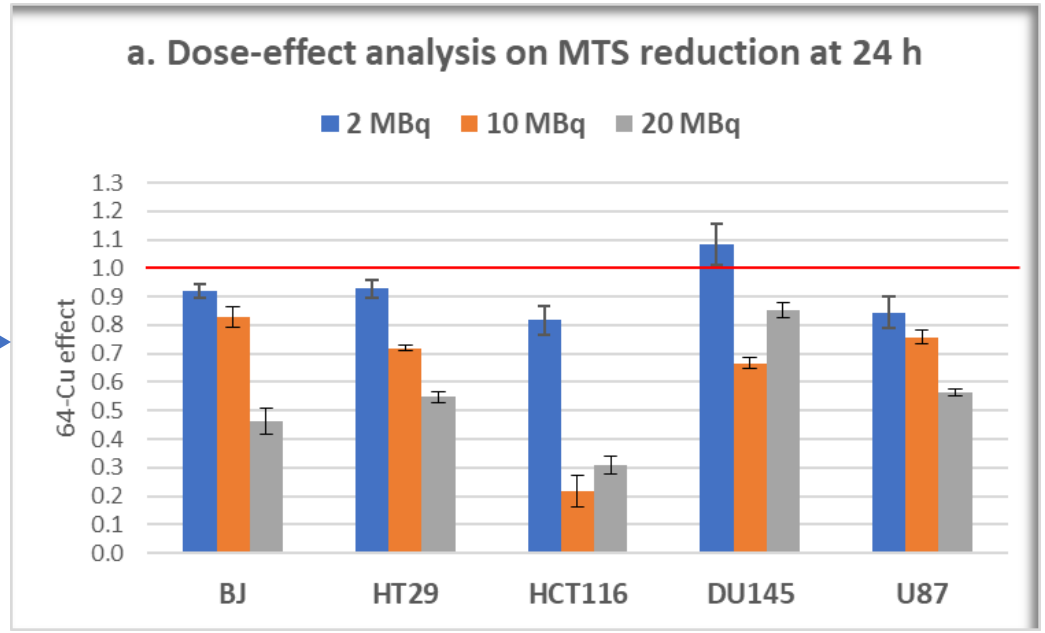
Unfolded Protein Response ATF4, ATF6, ATF6B, BBC3 (PUMA), BID, CALR, DDIT3 (GADD153, CHOP), DNAJC3, HSP90AA1, HSP90B1, HSPA4 (HSP70), HSPA5 (GRP78)

Inflammatory Response CCL2 (MCP-1), CD40LG, CRP, CXCL8 (IL8), IFNG, IL1A, IL1B, IL6, TLR4, TNF

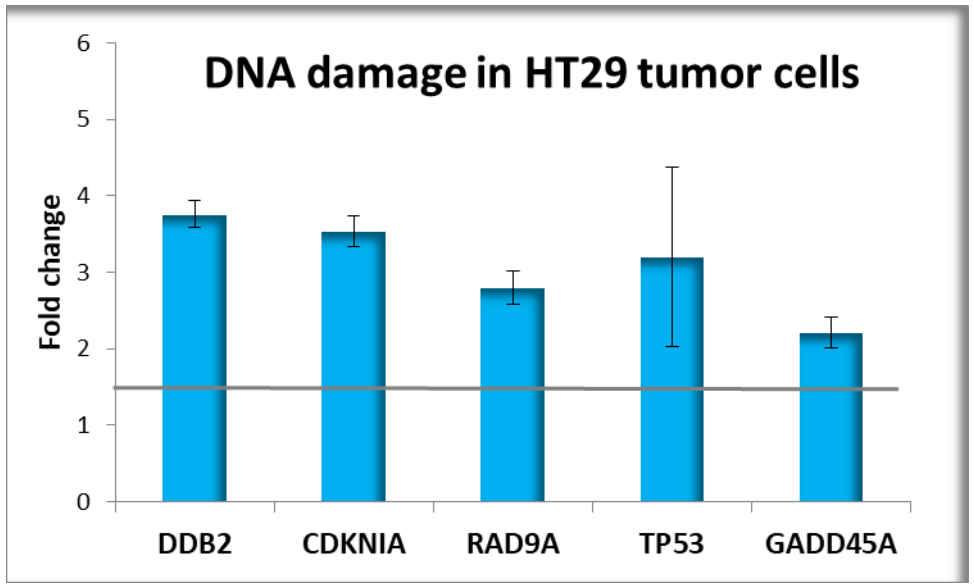


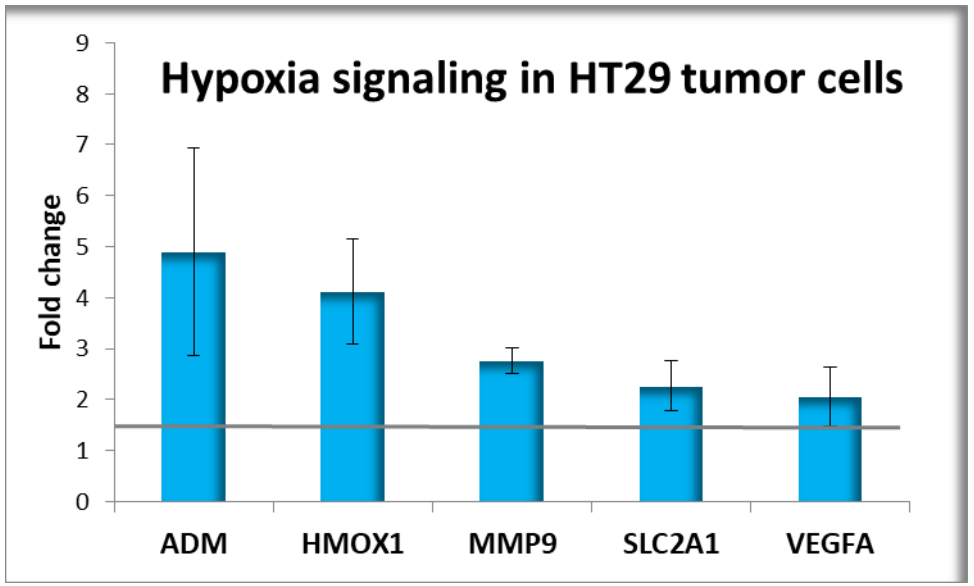
Treatment of cells with 20 MBq 64-Cu drastically reduced the number of viable cells in culture at 24 h after radioisotope addition, both in the case of tumor and normal cells. HCT116 tumor cells proved to be slightly more sensitive to the treatment than normal fibroblasts.

In the dose range 2-20 MBq/mL, most of the investigated cells showed a dose-dependent relationship. HCT116 tumor cells strongly reacted at 10-20 MBq/mL, irrespective of dose. The most notable differences between tumor cells and normal BJ fibroblast were registered at 10 MBq/mL.



Significant gene expression changes induced by 64-Cu (FC > 1.5) in HCT116 human colon carcinoma cells at 24 h after radioisotope addition





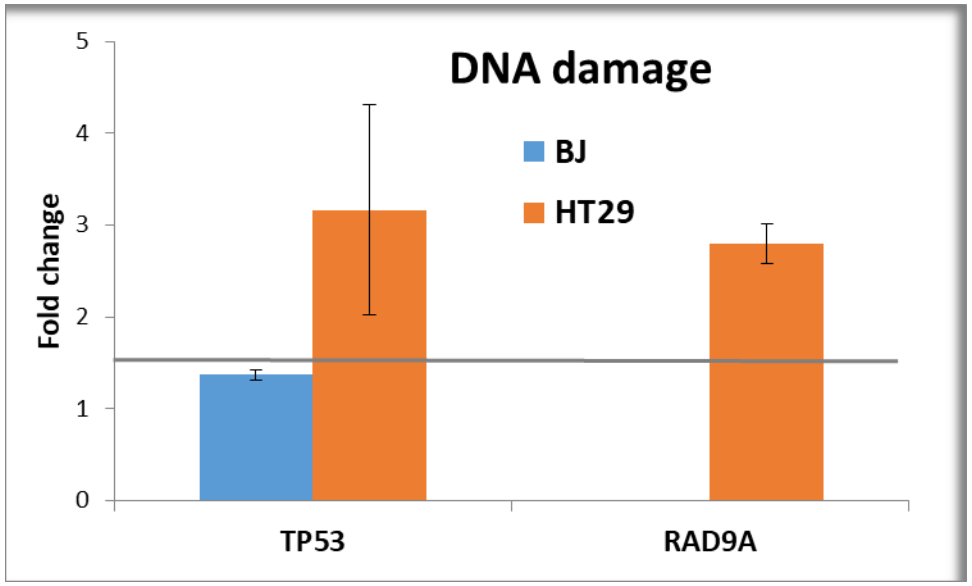
Regarding **redox genes**, only GSR was consistently overexpressed in HT29 tumor cells (FC=6.33 ± 3.92).

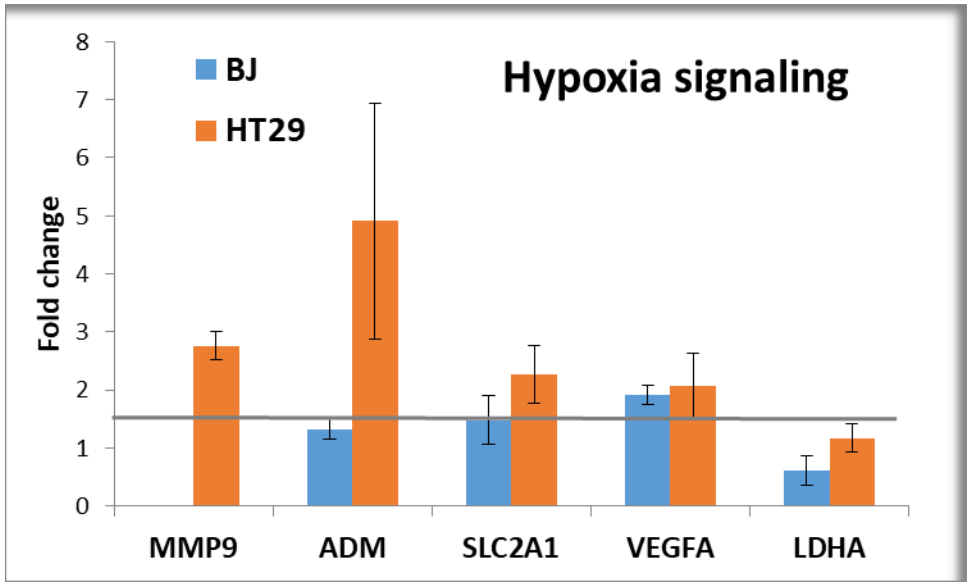
Gene expression data on cellular responses to stress highlighted that DNA damage and hypoxia signaling were triggered by the exposure of HCT116 cells to 64-Cu for 24 h.

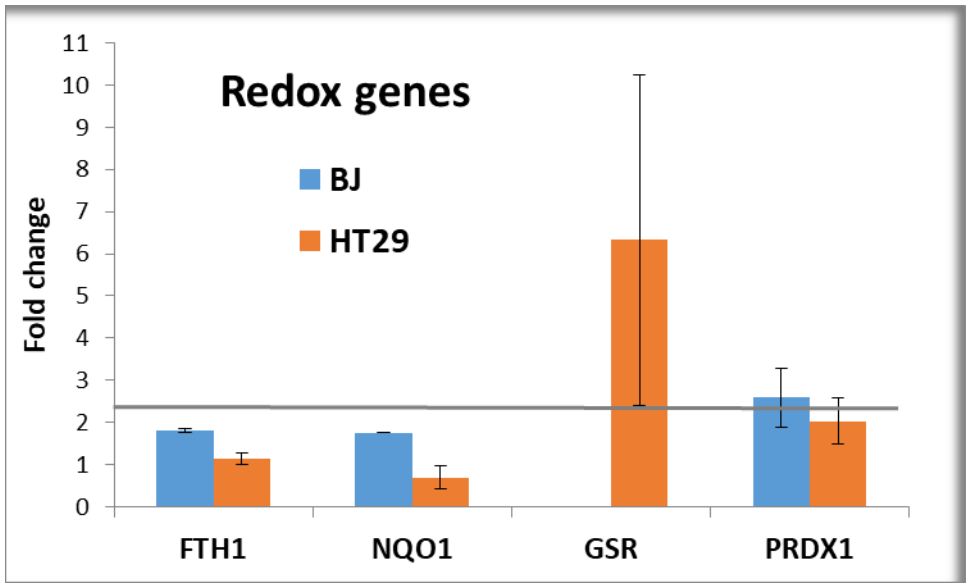
Protective cellular responses

- DDB2 is involved in DNA repair by nucleotide excision repair (NER), while CDKN1A triggers cell cycle arrest for allowing DNA damage repair. These protective responses may delay tumor cell death.
- Increased expression levels of the hypoxia-responsive genes ADM, HMOX1, SLC2A1and VEGFA are known to favor tumor progression.

Genes that are differentially expressed in HT29 tumor cells and normal BJ fibroblasts

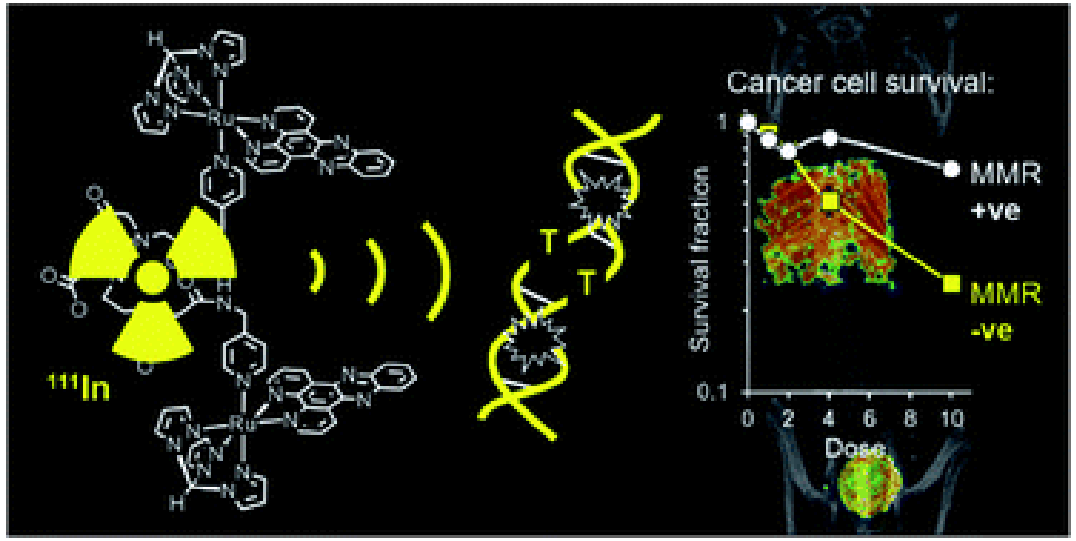






The increased expression levels of some protective genes in HCT116 tumor cells, as compared to normal BJ fibroblasts, corroborated with the drastic reduction of the number of metabolically active tumor cells, indicate that these protective mechanisms cannot counteract the death signals triggered by 64-Cu in tumor cells.

CONCLUSION



In vitro exposure of human normal and tumor cells to 64-Cu was shown to trigger genotoxic stress, along with hypoxia and oxidative stress, with distinctive patterns of gene expression.

Pharmacologic modulation of the identified pathways may enhance the therapeutic effect of 64-Cu in tumors, or may protect normal cells against the radiotoxic effect of 64-Cu.

Acknowledgements

Work was supported by the UEFISCDI through the grant PCCDI 64/2018, component project 3, and by the Ministry of Research, Innovation and Digitization through the NUCLEU grant PN 19.29.02.02/2019.