THE IMPACT OF ARGININE DEPRIVATION ON CANCER CELL VIABILITY AND SIGNALING PATHWAYS ASSOCIATED WITH HYPOXIA

CHORNYI S[2]., SHUVAYEVA G[1]., BOBAK Y[1,2]., STASYK O[1,2].

[1] Institute of Cell Biology, National Academy of Sciences of Ukraine; Drahomanov Str., 14/16, Lviv 79005, Ukraine

[2] Ivan Franko Lviv National University; Hrushevky Str., 4, Lviv 79005, Ukraine

contact: chornyi.serhii95@gmail.com

BACKGROUND

Deregulation of cell metabolism is a key event in the development and progression of cancer. For instance, one of the hallmarks of part of cancer cells is their auxotrophic for certain amino acids. This feature can be used to design selective anticancer therapies based on certain amino acid deprivation, for instance, arginine (Fig.1). For this purpose was used ADI-PEG20 (Pegylated arginine deiminase) or rhArg-PEG (Pegylated human recombinant arginase) to deplete the external supply of arginine, causing arginine-dependent cancer cells to stop proliferation or even die while leaving the patient's normal cells unharmed (Stasyk et al., 2015; Fultang et al., 2016; Savaraj et al., 2010). However, this approach is not effective enough. Also, some tumors develop resistance to Arg starvation through reactivation of ASS1 (Argininosuccinate synthetase 1) expression. The molecular mechanism of tumor cell response to arginine deficiency is not fully clarified (Tsai et al., 2017; Bobak et al., 2010).

Another important pathological feature of solid tumors is the development of hypoxia. An answer of tumors on hypoxia in many cases mediated through changes in gene expression regulated by the transcription factor HIF (Hypoxia-inducible factor). So it is very important to find out how changes in hypoxia-related signaling pathways will impact cancer cells under arginine deprivation.

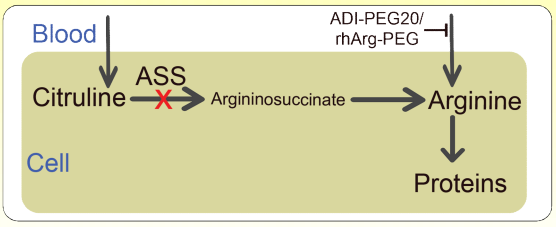

Fig. 1. Arginine deprivation therapy based on cancer cells ASS silencing.

METHODOLOGY

Colon cancer cell lines HCT-116, HT29 were cultured in the arginine-supplied (complete) (CM) or in the arginine-free medium (AFM). Hypoxic condition was mimic by addition of 0.1 and 0.2 mM cobalt (II) chloride ($CoCl_2$) (72h) that protect HIF-1α from degradation by inhibition of prolyl-hydroxylases (PHD) enzymes (the oxygen sensors) through replacement of Fe(II) making these enzymes unable to mark HIF-1α for degradation by von Hippel-Lindau (VHL) which is the substrate recognition component of an E3-ubiquitin ligase (Fig.2) (Zhang et al., 2007, Triantafyllou et al., 2006). MTT assay was used for measuring cell viability rates. Gene expression was determined by RT (reverse transcription)-PCR and Western blotting.

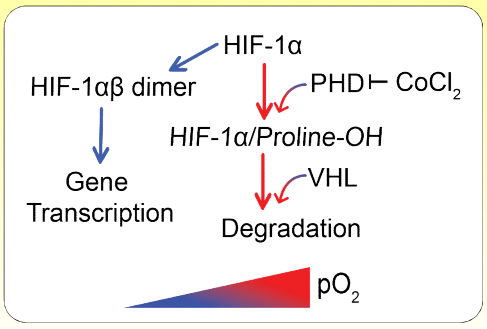

Fig. 2. Scheme of the mimic of the hypoxic condition by inhibition of ubiquitin-proteasomal degradation of HIF-1α.

RESULTS

First of all, we analyzed the impact of arginine starvation on the expression of genes associated with the hypoxic condition – Hif1α and VEGFA (Vascular endothelial growth factor A). In human colon cancer cell lines HCT-116 and HT29 arginine starvation did not induce any changes in the expression of transcription factor HIF1a under the influence of arginine deficiency. But this condition leads to the increase of the amount of VEGFA mRNA (Fig. 3), which can be partly explained by the role of neovascularization in supply of extracellular arginine.

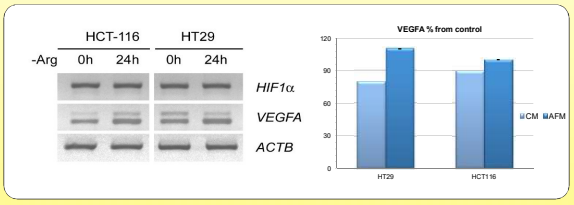

Fig. 3. Expression of HIF1a and VEGFA in cells under arginine deprivation.

After this, we performed artificial hypoxia with the application of $CoCl_2$ and analyzed the response of cancer cells on artificial hypoxia in arginine-free medium. We analyzed main signaling pathways associated with hypoxia under this condition. In the full medium we detected strong inhibition of p38 kinase caused by $CoCl_2$. This condition also did not cause any changes in mTORC1 activity, as shown by phosphorylation of S6 protein. But in arginine-free medium, no changes in p38 or mTOR was detected (Fig.4).

Arginine deprivation also associated with development of unfolden protein response (UPR) (Bobak et al,. 2016). So, we analyzed one of the main markers of UPR – GRP78 (78 kDa glucose-regulated protein). $CoCl_2$ exposure induced accumulation of GRP78, but, surprisingly, under arginine deprivation, effect of $CoCl_2$ was reversed.

We analyzed the impact of $CoCl_2$ on activation of apoptosis and cell prolifaration in our cell lines. No impact on caspase-dependent cleavage of PARP (Poly (ADP-ribose) polymerase) was detected (Fig.4). We detected decrease in cell proliferation rate caused by cobalt chloride in arginine-supplied medium. Under arginine deprivation, no changes were detected (Fig.5).

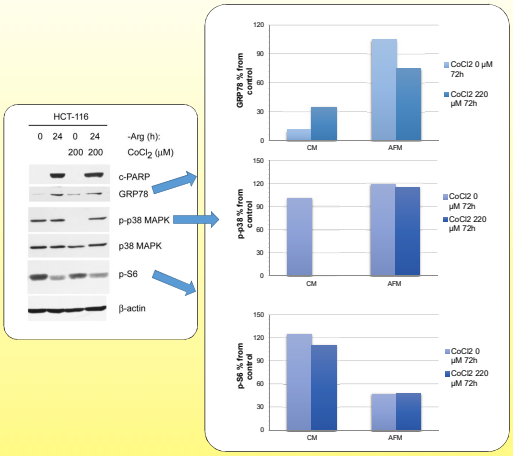

Fig. 4. Western blots of proteins associated with hypoxia and answer on arginine deprivation.

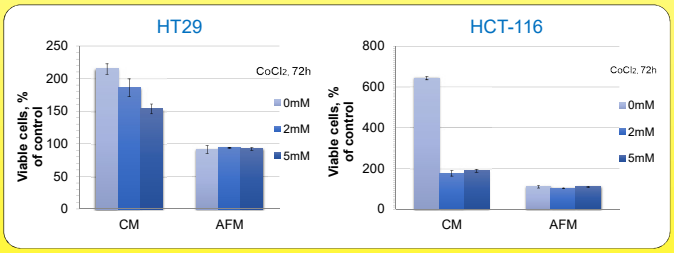

Fig. 5. Impact of $CoCl_2$ on cancer cell proliferation under arginine starvation.

Conclusions

The artificial enhancement of hypoxia did not increase the cytotoxicity of arginine deprivation towards the tested human cancer cells, but inhibits mTOR signaling pathway in arginine-supplied (complete) medium and reduce UPR induced by arginine starvation.

Involvement of HIF-1α turnover in mechanisms underlying arginine deprivation response demands further investigations.