

Prostate cancer cells are responsive to glucose and glutamine deprivation and ARHGAP21 has a promising role in the energetic metabolism through the regulation of Rho GTPases.

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Abstract

ARHGAP21 negatively regulates the Rho GTPases RhoA, RhoC and Cdc42, which have been recently associated with cell metabolism. Tumor cells present changes in metabolism and a higher uptake of nutrients such as glucose and glutamine. Therefore, cell metabolism has emerged as a potential therapeutic target. The objective of this work was to investigate the effects of glucose and glutamine deprivation in the survival of prostate cancer cells, and the participation of ARHGAP21, RhoA and RhoC.

Key words: ARHGAP21, cancer cell metabolism, Rho GTPases.

Introduction

It is known that changes in cell energy metabolism is essential for the establishment and progression of cancer [1], including prostate cancer. Tumor cells present high levels of glycolysis even in conditions of high concentrations of oxygen, which is known as the Warburg effect [2]. These cells uptake more glutamine in order to compensate the lower ATP production via glycolysis, in comparison to oxidative phosphorylation [3]. Therefore, glucose and glutamine pathways have emerged as important therapeutic targets. Rho GTPases are cytoskeleton regulators that have been recently associated to cell metabolism [4]. ARHGAP21 is a RhoGAP that negatively regulates RhoA, RhoC and Cdc42 and is involved in tumor development [5]. The aim of this study was to investigate the effects of glucose or glutamine reduction in the survival of two prostate cancer cell lines. Moreover, we evaluated the participation of ARHGAP21 and Rho GTPases in cell resistance to glutamine deprivation.

Results and Discussion

LNCaP and PC3 cells were cultured under different glutamine and glucose concentrations for 72 hours and mitochondrial activity was evaluated through MTT, whereas cell proliferation, apoptosis and autophagy were analyzed by flow cytometry. ARHGAP21, p62, Beclin, LC3 and Caspase 3 expressions were evaluated using western blot. ARHGAP21, RhoA or RhoC knockdown were performed using specific siRNAs. Mitochondrial activity and proliferation of both cells were strongly decreased under progressive glucose reduction and a minor effect was observed by glutamine removal. Glucose reduction increased acid vesicles in LNCaP cells and autophagic proteins in LNCaP and PC3 cells. Glutamine reduction also induced the expression of autophagic proteins in LNCaP cells. Very low levels of apoptosis and caspase activity were observed upon glucose or glutamine deprivation.

Interestingly, ARHGAP21 was downregulated by glucose and glutamine reduction in both cell lines, especially by glutamine. The downregulation of ARHGAP21 indicates its participation in the response triggered by the absence of both nutrients, possibly through the regulation of Rho GTPases. ARHGAP21 knockdown did not alter cell survival in the tested conditions. However, the silencing of RhoA and RhoC reduced mitochondrial activity and an additional effect in the decrease of mitochondrial activity was observed upon glutamine deprivation and RhoC knockdown.

Conclusions

Our results indicate that both cell lines respond to glutamine and glucose deprivation through a cytostatic and not apoptotic effect. ARHGAP21 might participate in the resistance to glucose/glutamine removal, through the regulation of RhoA/C. Moreover, RhoC silencing maybe an additional strategy to decrease glutamine deprivation resistance in cancer cells.

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⁴ Wilson KF et al. Rho GTPases and their roles in cancer metabolism. *Trends Mol Med*. 2013 Feb; 19(2):74-82. Epub 2012 Dec 5.

⁵ ARHGAP21 is a RhoGAP for RhoA and RhoC with a role in proliferation and migration of prostate adenocarcinoma cells. Lazarini M et al. *Biochim Biophys Acta*. 2013 Feb;1832(2):365-74.