Research & Reviews: Journal of Microbiology and Biotechnology

e-ISSN: 2320-3528
p-ISSN: 2347-2286

A Breakthrough In Extracellular ATP-Driven Invasion Process

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COMMENTARY

Received date: 27/07/2020
Accepted date: 14/08/2020
Published date: 21/08/2020

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Keywords: ATP, HIF signaling; cancer, Invasion

COMMENTARY

Recently, an original article titled "Extracellular ATP promotes breast cancer invasion and epithelial-mesenchymal transition via hypoxia-inducible factor 2α signaling" was published in Cancer Science [1]. In this article, we firstly demonstrated that extracellular ATP could up-regulate both HIF-1/2α and HIF signaling related genes even under normoxia conditions. Further, using transwell invasion assay, we demonstrated that it was HIF-2α that mediated ATP-driven invasion. In addition, we illustrated that ATP could regulate HIF-2α via P2Y2-AKT-PGK1 pathway, provoking HIF-2α target genes, through which LOXL2 and MMP-9 mediated ATP-driven invasion, and E-cadherin and Snail mediated ATP-induced epithelial-mesenchymal transition (EMT). We also demonstrated the function of HIF-2α in promoting tumor growth and metastasis via xenograft model. Moreover, molecules involved in ATP-HIF signaling were associated with clinical breast cancer progression, indicating that the ATP-HIF signaling might be a potential target for breast cancer therapy (Figure 1).

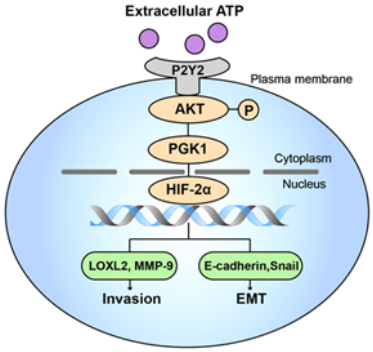

Figure 1. The ATP- HIF-2α signaling hypothesized by Yang et al. [1]. In this model, ATP could up-regulate HIF-2α by P2Y2-AKT- PGK1 signaling, provoking HIF-2α targets, among which LOXL2 and MMP- 9 mediate ATP-driven invasion, and E-cadherin and Snail mediate ATP- driven EMT.

e-ISSN: 2320-3528
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INTRODUCTION

Extracellular ATP accumulates at tumor sites

ATP is the most common energy molecule in human cells, mediating various biological functions [2]. Extracellular ATP is negligible in healthy tissues [3,4] while accumulating at high levels in tumor sites [5,6] and possibly reaching hundreds of μmol/L in tumor microenvironment (TME) [4,5]. Extracellular ATP in tumor microenvironment is mainly released from dead tumor cells [6,7]. As early as 2008, using plasma membrane luciferase, Pellegatti et al. reported that the concentration of ATP in the tumour interstitium is in the hundreds micromolar range, or even possibly higher, as the signal saturated above 700 μM ATP [8]. In addition, using the pmeLUC probe, Virgilio et al. unequivocally demonstrated that at sites of tissue damage, inflammation,TME and tumor metastases, extracellular ATP could be as high as hundreds micromolar as well [7]. In recent years, all techniques used measuring the concentration of extracellular ATP had provided the same answer: ATP level in healthy tissues is very low whereas in stimulated or diseased tissues it could reach hundreds μmol/L, making a great impact on tumor biology [9,10].

Previous study of extracellular ATP in cancer

Our team was the first to demonstrate the pro-invasive function of extracellular ATP in cancer [11,12]. In 1992, WG Fang reported that extracellular ATP could inhibit the growth of androgen-independent prostate carcinoma cells via P2-purinergic receptor. They showed that 100μM ATP stimulation resulted maximal Ca2+ mobilization in prostate cancer cells, causing downstream signal transduction [12]. After that, our team began to investigate the downstream effectors involved in ATP signaling and had made great progressions on the mechism involved in ATP-driven invasion. In 2005, Chen et al. reported that extracellular ATP could activate ERK1/2 and p38 protein kinases signaling via G protein-coupled P2Y purinoceptors, promoting prostate cancer cell invasion [11]. This work provided important clues that the downstream signaling of extracellular ATP-P2 receptors contributed to cancer cell invasion. Further, to investigate the target genes that mediate ATP-driven invasion process, Li et al. performed cDNA microarray using P2Y2-siRNA treated prostate cancer cells, they found that ATP could promote IL-8 and Snail expression while inhibiting E-cadherin and Claudin-1 levels via P2Y2 receptor, regulating tumor EMT and invasion [13]. And they also demostrated the cooperation of P2Y2 receptor and EGFR promoted prostate cancer cell invasion via ERK1/2 pathway [14]. It is known that P2X7 receptor is also an important channel highly expressed in cancer cells and mediates multiple tumor biological functions [15,16]. Using P2X7-siRNA treated prostate cancer cells, Qiu et al. illustrated the remarkably alterations of EMT/invasion-related genes Snail, E-cadherin, Claudin-1, IL-8 and MMP-3, as well as PI3K/AKT and ERK1/2 signaling, all of which were vital in cancer invasion and metastasis [17].

In the recent years, we found that besides prostate cancer, extracellular ATP also played an important role in breast cancer cells. In 2017, Zhang et al. reported the stimulation of Wnt signaling as well as its downstream target genes CD44, c-Myc and cyclin D1 via ATP-P2Y2 signaling, promoting breast cancer cell invasion both in vitro and in vivo [18]. In 2018, Liu et al. began to explore the function of extracellular ATP both on tumor cells and surrounding fibroblasts, both of which are considered as a major part in TME [19]. As a result, we found that apart from stimulating breast cancer cell motility via up-regulating intracellular S100A4 in cancer cells, ATP also enhanced the ability of breast cancer cells to transform fibroblasts into cancer-associated fibroblast (CAF)-like cells, which in turn secreted S100A4 to further promote cancer cell motility. This work linked the interactions between breast cancer cells and fibroblasts in ATP treatment and firstly demonstrated that it is their cooperation that promoted breast cancer invasion and metastasis.

Although the mechanism of extracellular ATP in promoting tumor invasion has already been studied, however, there are still multiple genes or signalings involved, which need further investigation.

Previous study of HIF-2α- mediated tumor invasion

Hypoxia-inducible factor 2α (HIF-2α) is a member of a basic-helix-loop-helix/PAS domain containing transcription factors, showing a high homology to hypoxia-inducible factor 1α (HIF-1α) [20]. It is well established that HIF-1α primarily mediates fast (acute) response and HIF-2α mediates late (chronic) response to hypoxia [21]. Both HIF-1α and HIF-2α could transactivate genes in response to hypoxia stress and mediate tumor invasion process through activating different target genes [22,23]. The function of HIF-1α had been well-characterized previously whereas no exclusive HIF-2α signalings had yet been identified, and the remarkably impact of HIF-2α in tumor is growing a relatively new topic in recent years [21]. In addition, evidence showed that the level of O2 could determine partner proteins and target genes of HIFs [24], indicating the regulation of HIF-2α signaling could even vary under hypoxia and normoxic conditions. Based on our previous results, KEGG pathway analysis showed that HIF-2α signaling was enriched in ATP treatment without hypoxia. And the regulation of HIF-2α signaling in normoxic conditions is less clear, which needs further exploration as well.

PERSPECTIVES

A new link between extracellular ATP pro-invasive function and HIF signaling

Writing in this paper, Yang et al. firstly demonstrated that extracellular ATP could stimulate HIF signaling under normoxic conditions [1]. Using cDNA microarray, we found that HIF2A and other target genes involved in HIF signaling could be regulated

e-ISSN: 2320-3528
p-ISSN: 2347-2286

in 24 hr ATP treatment. In fact, it is worth noting that HIF-1α is transiently stabilized and primarily mediates acute responses, whereas HIF-2α is gradually accumulated and governs prolonged gene activation [21]. We hypothesized that HIF-1α might be up-regulated via extracellular ATP in some indicated time points as well, perhaps earlier than 24 hr ATP treatment. Indeed, we proved that extracellular ATP could activate both HIF-1α and HIF-2α signaling at different time points under normoxic conditions, providing important clues that there is a new link between ATP signaling and HIF signaling without hypoxic stress.

In fact, the HIFs are frequently coexpressed in tumor [25], thereby raising the question that what their specific roles in ATP-mediated tumor invasion and metastasis. The answer lies not only in which genes are transcribed by HIF-1α or HIF-2α, but in the conditions under which HIF-1α and HIF-2α are stabilized, transcriptionally active, and subsequently utilized by hypoxia-regulated genes [21,26]. Another novel finding in our research was that although both HIF-1α and HIF-2α were in response to ATP treatment, we firstly demonstrated it were HIF-2α and its targets genes that mediated ATP- driven EMT and invasion function. It is worth noting that evidence suggested that the invasive and metastatic activities in vivo are complex processes, including the loss of cell-cell adhesion in primary tumor, migration, invasion and implantation to form the secondary tumor[27]. Therefore besides transwell invasion assay, we also investigated EMT process, both of which provided solid proofs that HIF-2α functioned in ATP- driven tumor invasion.

New mechanism of HIF signaling regulated via extracellular ATP

In this paper, using sliver staining and mass spectrometry, we identified PGK1 protein could interact with HIF-2α under normoxic conditions. Further, we demonstrated that PGK1 could regulate HIF-2α as well, which was interesting, as PGK1 has been shown to be a HIF-1α-target gene in many cells [28,29]. And we proved that it was AKT signaling, not HIF-1α, that mediated PGK1- HIF-2α in ATP treatment. In fact, there was research of our team had already revealed the involvement of AKT signaling in bFGF-driven HIF-1α activation under normoxic conditions as early as 2005 [30]. Here we want to explain the results that because HIF-1α and HIF-2α have 48% amino acid identity and similar protein structures, hence they could display both unique and overlapping patterns in molecular signaling pathways [31]. In summary, both work of our team had provided important guidance for the mechanism involved in HIF signaling, paving the way for clarifying the regulation of HIFs under normoxic conditions, the field not well-studied now.

New findings of ATP- HIF-2α target genes

HIFs had been directly or indirectly linked to the regulation of most investigated hypoxia-induced genes. Previous research worlwide had validated HIF-2α-regulated genes at some different O2 series. For example, Holmquist-Mengelbier et al. proved that SERPINB9 could function as a HIF-2α target gene at 5% oxygen, which was associated with metastatic melanoma and predicted poor prognosis in anaplastic large cell lymphoma [21]. Besides, there were also multiple genes that functioned as target genes of HIFs as hypoxia responsive, such as DEC1/BHLHB2, NDRG1, STC1, VEGF and TRIO [32-34]. However, few studies addressed the HIFs at near-physiological oxygen tensions.

In this paper [1], we foucused on the ATP- driven process and demonstrated the directly binding of HIF-2α to the promoter of invasion- associated genes LOXL2 and MMP-9 and EMT- associated genes E-cadherin and Snail, directly and unequivocally showed that HIF-2α was highly involved in regulation of ATP- driven process at physiological oxygen tensions. Indeed, both HIF-2α and its tagets genes we found were associated with clinical breast cancer progression, suggesting the signaling of ATP- HIF-2α-target genes in our reseach might provide vital targets for clincial breast cancer therapy in the near furture.

Limitations and future applications

The limitation of our research was that we had not investigated the detailed mechanism how P2Y2 receptor mediated ATP-HIF-2α signaling. In fact, there are several P2 receptors could mediate extracellular ATP-driven invasion, such as P2X7 [17]. And how could P2Y2 receptor regulate the downstream HIF signaling and whether there were other receptors involved in the process were unclear, which still need additional experiments to explore this ATP-P2 receptor-HIF signaling. Here we consider the most important novelty in our article is the discovery of ATP-HIF signaling and we will continue to investigate the P2 receptors involved in the near future.

Despite the limitations in this research, we had made a breakthrough in extracellular ATP- driven invasion process.

CONCLUSION

In summary, we are the first to demonstrate the link between extracellular ATP and HIF signaling under normoxic conditions while illustrating the mechanism involved, including HIF-2α interacted protein and target genes. And mechanism in this paper will undoubtedly enhance the understanding of HIF signaling, and the targets in ATP- HIF-2α axis will shed a light on clinical breast cancer therapy.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

e-ISSN: 2320-3528
p-ISSN: 2347-2286

CONCLUSION

This work was supported by grants to Xin-Xia Tian and Wei-Gang Fang from the National Natural Science Foundation of China (Nos. 81872382 and 81621063).

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e-ISSN: 2320-3528
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