Sci. Bull. (2015) 60(24):2120–2128
DOI 10.1007/s11434-015-0950-5

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Article

Life & Medical Sciences

1 T moderate intensity static magnetic field affects Akt/mTOR pathway and increases the antitumor efficacy of mTOR inhibitors in CNE-2Z cells

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Received: 21 September 2015 / Accepted: 10 November 2015 / Published online: 16 December 2015

Abstract Static magnetic field (SMF) has been known to affect cell proliferation in a cell-type-dependent manner, while the mechanism still remains unclear. We found that 1 T moderate intensity SMF inhibits cell proliferation of nasopharyngeal carcinoma CNE-2Z cells and the Akt/mTOR signaling pathway, which is upregulated in many cancers. mTOR inhibitors are potential chemodrugs, but their clinical effects are limited by the feedback reactivation of other signaling components such as EGFR and Akt. We showed that 1 T SMF increases the antitumor efficacy of mTOR inhibitor Torin 2. In addition, 1 T SMF increases the inhibition efficiency on mTOR substrates phosphorylation and represses the mTOR inhibitor-induced feedback reactivation of EGFR and Akt. Our study not only demonstrates that mTOR/Akt pathway is one of the molecular targets of SMFs in cells, but also reveals the clinical potentials of combinations of mTOR inhibitors and SMFs in cancer treatment.

Keywords Static magnetic field · Cell proliferation · mTOR · mTOR inhibitor · Feedback reactivation

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1 Introduction

With the development of modern appliances, including MRI machines in the hospitals, there are increasing concerns about the potential impact of magnetic fields on human health. Therefore, it is very important to study the cellular effects of magnetic field to help address this issue. However, various experimental parameters such as cell types, magnetic field types as well as other parameters including the exposure time, magnetic field intensity, and frequency have sometimes led to contradictory results, which prevented people from understanding the mechanisms of biological effects of the magnetic field correctly.

Multiple studies show that cells respond differently to magnetic fields with different types and intensities. For example, Grassi et al. [1] used 50-Hz, 1 mT pulse magnetic field to stimulate rat pituitary GH3 cells and found that the cell proliferation was increased. However, Rosen and Chastney [2] used 0.5 T static magnetic field on GH3 cells and found the cell proliferation was obviously inhibited. Pulsed electromagnetic fields have variable parameters including intensity and frequency, which makes it difficult to study the biological mechanisms of magnetic effects comprehensively and systematically. It was shown that pulsed magnetic fields with different frequencies can have diverse effects on cell proliferation [3]. In comparison, static magnetic fields have fewer variables which confer them obvious advantages to study the magnetic effects at cellular and molecular levels. It is interesting that SMFs seem to be able to inhibit cancer cell growth [2, 4–7] but not non-cancer cells [5, 6, 8–10]. However, the molecular mechanisms underlying this cell-type-dependent difference between cancer and non-cancer cells responding to static magnetic fields are still not clear.

PI3K/Akt/mTOR is one of the important signaling pathways that are frequently deregulated in cancers [11, 12]. Mammalian target of rapamycin (mTOR) kinase is a key regulator that incorporates growth factors, nutrients, energy signals into regulation of cell proliferation, and growth in cells [13, 14]. It binds to two different sets of proteins to form mTORC1 (the mTOR complex 1) and mTORC2 (the mTOR complex 2), in which mTORC1 is sensitive to rapamycin (also called sirolimus) while mTORC2 is not [15–19]. Other inhibitors that inhibit both mTORC1 and mTORC2 are also developed, such as Torin 1, Torin 2, and AZD8055 [20–24]. The most prominent substrates of mTORC1 are S6K1 and 4EBP1, while the major substrate of mTORC2 is Akt at S473 [18]. It was shown that mTOR pathway involves in a series of negative feedback loops which dynamically change the activation state of factors along its signaling. For example, mTOR inhibitors can cause the negative feedback loop to reactivate its upstream components, which might be one of the reasons for the limited efficiency of their clinical application [25–27]. Actually, combining mTOR inhibitors with EGFR inhibitors is shown to result in an improved antitumor efficacy because EGFR inhibitors can repress the feedback loop-caused reactivation.

In this study, we report that 1 T SMF reduces cell proliferation of human nasopharyngeal carcinoma cells CNE-2Z and inhibits the Akt/mTOR pathway. In addition, SMFs increase the efficacy of mTOR inhibitors and repress the mTOR inhibitor-induced feedback reactivation of EGFR.

2 Materials and methods

2.1 Cell culture

CNE-2Z cells were cultured in RPMI-1640 supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin and streptomycin (P/S), 5 % CO_2, 37 °C. They were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

2.2 Reagents

The Torin 2 was a gift from Qingsong Liu's group in Chinese Academy of Sciences. The AZD8055 and rapamycin were from Shanghai Haoyuan Chemexpress. All phospho-specific antibodies, Akt, S6K, 4EBP1, and the HRP-linked anti-rabbit and anti-mouse IgG antibodies were from Cell Signaling Technology. The mouse monoclonal antibody for beta-tubulin was from Beijing TransGen Biotech. Pre-stained Protein Ladder (26616) and M-PER buffer were from Thermo Pierce. Protease inhibitor and phosphatase inhibitor cocktails were from Roche, and the PVDF membrane was from Millipore.

2.3 Cells treated with static magnetic fields and mTOR inhibitors

The magnets were from China Dafeng Zhongxin Permanent Magnet Material. To ensure the proper cell culturing conditions, the magnets were placed in the 37 °C CO_2 cell incubator. CNE-2Z cells were plated in 35-mm plates, which were placed right on top of the magnets (Fig. 1). The intensities of SMFs were measured with a Gauss meter. The max intensity of the magnet is in the center, where the cell plate was placed. 3–4 $\times$ 10^5 CNE-2Z cells were plated in each plate and treated with magnetic field for different time points without medium change.

For inhibitor treatment, CNE-2Z cells were plated in 24-well plates (1–2 $\times$ 10^5 cells per well) on the first day for cells to attach. Then, cells were treated with mTOR inhibitors or mTOR inhibitors combined with 1 T magnetic field for 3 d. Images were taken by Leica DMI4000B microscope. Experiment were repeated for at least three times.

2.4 Western blotting analysis

Cells were grown in 35-mm plates or 24-well plates and lysed by 200 or 100 μL of M-PER buffer supplemented with protease and phosphatase inhibitor cocktail at 4 °C for 20 min. For Western blotting, the whole cell lysate was mixed with 2X SDS loading buffer, boiled, and subjected to the SDS-PAGE in Bio-Rad Mini-PROTEAN Tetra Cell and transferred by Thermo Scientific Owl VEP-2 (7351). The PVDF membrane was blocked with 5 % NFDM (no-fat dry milk) at room temperature for 1 h. Corresponding primary antibodies were diluted in AbDil-Tween (TBS supplemented with 2 % BSA and 0.1 % Tween-20). HRP-conjugated secondary antibodies were diluted in TBS with 0.1 % Tween-20 and 5 % NFDM. Western blotting results were obtained by Bio-Rad *ChemiDoc*^{TM} *XRS+ System* and Beijing Tanon Fine-do X6. ImageJ software was used to quantify the protein relative level shown by Western blot.

2.5 Flow cytometry for cell cycle

Cells were harvested by trypsinization and washed with ice-cold PBS. The cells were then fixed in 70 % ice-cold ethanol (prechilled at −20 °C) and incubated at 4 °C for overnight. On the day of flow cytometry experiment, cells were spun down, washed with PBS, and stained in 50 μg/mL PI (propidium iodide) (Sigma) + 0.5 mg/mL RNAse (Sigma) in PBS + 0.5 % Triton-X100 for 30 min at room temperature and moved to 4 °C until time of analysis. Flow