

*Original Research Article*

# Depletion of nutrients stimulates further propagation of cancer cells

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Abstract

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In present study, B16-F10 cell line was grown in 96-well culture plate with different amount of culture medium. We expected the cell proliferation rate decreased accompanied with lower volume of medium. Interestingly, in 100  $\mu$ l culture medium, cell number of B16-F10 cells was significantly increased. This result indicated that low nutrient condition would not inhibit cell proliferation, but stimulated cell growth. To further confirm this notion, STO cell was cultured under different amount of culture medium. The result was in line with B16-F10 cells, growth rate of STO cells was significantly increased in 80  $\mu$ l. Above result suggested such depletion of nutrients stimulates further propagation of cancer cells. Cancer could be considered as a response outcome when cells strive to survive against stress. In present study, cells were grown with higher proliferation rate in lower supplement of culture medium. This data suggested that stress could stimulate the propagation of cells by lowering the nutrients of cell culture medium. This observation proposes a cancer formation mechanism, which provides a new research direction of cancer treatment. The stress factors that might cause tumorigenesis are diverse, and perhaps are not easy to remove. Finding a method or medicine that could inhibit cell proliferation under stress provides an opportunity to develop the key indicators and a method to effectively prevent cancer formation.

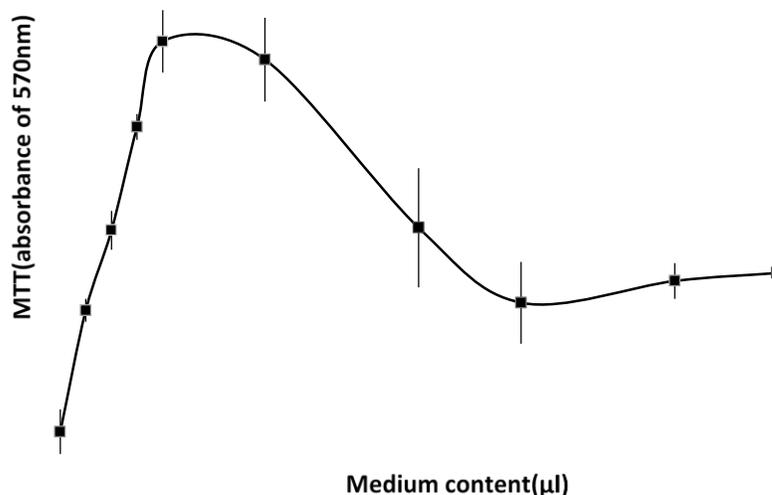
**Keywords:** B16-F10 STO, Nutrients, Propagation, Stress

## INTRODUCTION

Cancer is the leading cause of death and its therapy is among of the most important issue worldwide. Tumorigenesis is a process caused by multifactorial-mediated cell transformation including carcinogen, heavy metal, radiation and physical/chemical stimulation (Davis et al., 2013; Agrawal et al., 2013). Not only carcinogens, several cancers are caused by inherited genetic background. To date, the main clinical treatment relies on chemotherapy or protein specific targeting therapy, but there is no effective therapy for cancer (Benigni and Bossa, 2011). In the process of biological evolution, stress response plays an important role in the survival of organisms via cell reproduction and metastasis. Although humans are multicellular organisms, each cell could be

regarded as an individual organism. Thus single cell also has similar post-evolutionary response to stress. During evolution, stress response is a key factor that is required for organism to survive. For instance, excessive cold or heat, toxic chemicals or radiation, ultraviolet rays or electromagnetic pressure causes many biological reactions; one of which is the proliferation of reproductive reaction. These processes accelerate or start the reaction to the continuation of the presence of reproductive life.

In animals, fully differentiated cell itself has an independent and complete genome, and can be considered as an independent life which can survive alone under the cell culture medium. Differentiated cells are also possible to adjust their lives through regulation of gene



**Figure 1. Cell growth of B16-F10 cells under indicated volume of medium.** Aliquots of B16-F10 cells were cultured in indicated volume of medium for 48 hours, and cell proliferation was quantified by MTT assay.

expression. Genes which response to stress response are conserved through thousands of years of evolution with the best suited to their survival. Once the stress generated, the cell starts to express genes required for reproduction, termed proliferation. However, it would be diversified while cells start to enter propagation status. In the case of transformed, tumor or cancerous cells, there are few barriers to proliferation and a subsequent sub-toxic oxidant dose which may provide a further stimulus for pronounced proliferation (Aw, 1999). Cells without inhibition would replicate through different ways, this is a complex adaptive evolution for survival. And lack of nutrition is possible to be one of stresses to induce self-regulated gene expressions and can transfer cells into a state of cell propagation.

In present study, we observed that tumor cell increased the proliferation rate under lower nutrient supplement. This result therefore supports a notion for cancer formation, and possibly provided the strategy for cancer therapy. Further, the consequence of tumor growth worse those cancer patients' health condition and cause death. It is therefore crucial to find out the key factor, which senses stress and triggers stress response.

## MATERIALS AND METHODS

### Cell culture

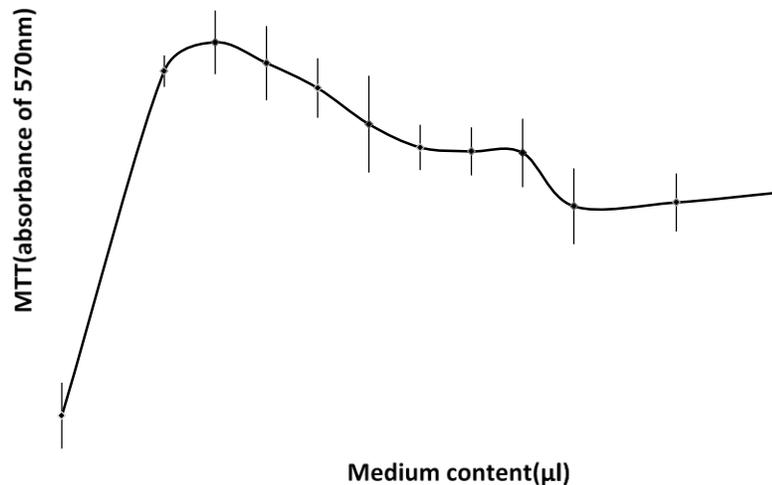
B16-F10 (mouse melanoma metastatic cell line) or STO (mouse embryonic fibroblasts) cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) with 10% fetal bovine serum (FBS, Gibco BRL), 100 U/mL penicillin (Gibco BRL), and 100 mg/mL streptomycin (Gibco BRL) at 37 °C in 5% CO<sub>2</sub> and 100% humidity.

### Cell proliferation

Cell proliferation of B16-F10 or 3T3 was estimated in a 24-well plate with 104 cells per well. The cell density of B16-F10 or 3T3 in of culture medium were analyzed after two days by an assay based on the reduction of the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT). Briefly, the cells were incubated with fresh medium containing 1.5 mg/ml MTT for 1 hour. The cells with functional mitochondria can reduce MTT to an insoluble purple formazan product. The medium was then aspirated and the reduced formazan was dissolved with dimethyl sulfoxide (DMSO, ---). The optical density (OD 570) was measured using a Dias Microwell Plate Reader and the absorbance was taken as proportional to the number of viable cells.

## RESULTS

In present study, B16-F10 cell line was grown in 96-well culture plate with different amount of culture medium. We expected decrease in the cell proliferation with lower volume of culture medium. Interestingly, in 100μl culture medium, cell number of B16-F10 cells was significantly increased (Figure 1). This result indicated that low nutrient condition would not inhibit cell proliferation, but stimulated cell growth. To further confirm this notion, STO cell was cultured under different amount of culture medium. The result was in line with B16-F10 cells, growth rate of STO cells was significantly increased in 80 μl (Figure 2). Above results suggested that depletion of nutrients stimulates further propagation of cancer cells.



**Figure 2. Cell growth of STO cells under indicated volume of medium.**  
Aliquots of B16-F10 cells were cultured in indicated volume of medium for 48 hours, and cell proliferation was quantified by MTT assay.

## DISCUSSION

Cancer could be considered as a response outcome when cells strive to survive against stress. In present study, cells were grown with higher proliferation rate in lower supplement of culture medium. This data suggested that stress could stimulate the propagation of cells by lowering the nutrients of cell culture medium. This observation proposes a cancer formation mechanism, which provides a new research direction of cancer treatment.

Due to the mass propagation of end-stage cancer cells, some cancer cells are deprived of nutrients. Changes in nutrient supply and oxygenation of cell cultures by altered culture media volumes has been shown to influence metabolic rates and levels of glycolytic enzyme activities (Gstraunthaler et al., 1999). Further, lower nutrient condition causes metabolic switch from oxidative phosphorylation (respiration) to glycolysis, which is called Warburg effect (Warburg, 1956). In previous study, nutrient deprivation would also accompany with Reactive oxygen species (ROS) production and induce the Warburg effect through ROS/ AMP-dependent protein kinase (AMPK)-dependent pathway (Wu et al., 2013). Hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ ), a master transcription factor to induce the Warburg effect through HIF-1 $\alpha$  accumulation, stimulates cell proliferation and survival (Warburg, 1956; Lee et al., 2004; Semenza, 2010). Meanwhile, ROS were shown to amplify HIF-1 $\alpha$  stabilization by inhibiting prolyl hydroxylase (Niecknig et al., 2012). Glucose is frequently metabolized through glycolysis. Cancer cells utilize glycolysis, which yields less ATP and can occur in hypoxic tissues which cannot obtain ATP through respiration (Warburg, 1956). Proliferating cells also have an increased uptake of glucose and glutamine (Vander Heiden et al., 2010). Glycolytic

metabolism switch has been reported not only promotes proliferation of cancer cells, but also protects them from cell death (Colell et al., 2007; Ferraro et al., 2008).

To evaluate whether the stress response is correlated with HIF-1 alpha or other oncogenes which cause the propagation of cancer cells; further studies which focus on whether the propagation of cancer cells under stress will be suppressed by inhibiting the activation of these candidate factors may give hopes to finding a novel treatment strategy for end-stage cancer patients. Production and removal of ROS is intimately linked to respiration and glucose and glutamine metabolism. ROS have a dual role in cancer, as signaling molecules that promote proliferation or as mediators of cell death induced by chemotherapy or ischemia (Sarsour et al., 2009; Coriat et al., 2011; Thomas et al., 2011). It is therefore crucial to identify the first factor which senses stress and triggers stress response. Inhibition of this stress-sensing factor may contribute to development of new cancer treatment.

## CONCLUSION

Finally, the stress factors that might cause tumorigenesis are diverse, and perhaps are not easy to remove. Finding a method or medicine that could inhibit cell proliferation under stress provides an opportunity to develop the key indicators and a method to effectively prevent cancer formation.

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