

Interaction effects between C2C12 and Walker 256 cells: In vitro Model of tumor effects in muscle tissue (C2C12 myotubes).

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Abstract

Cancer is the second cause of death in the world and can affect the host metabolism, increasing the muscle spoliation and decreasing the adipocyte tissue, inducing the cachectic state in these patients. This work aimed to analyse how the factors produced by tumour cells could affect the myotube C2C12 growth or activities when these cells were treated with culture medium from tumour cells; with this procedure, we could mimic the same situation as in cancer patient. The results showed that there is a correlation between the cellular area variation and the time of the treatments, and the W group led to a decreased cell confluence layer, suggesting that the tumour cells medium likely contained some factor that affected the C2C12 growth.

Key words:

Cancer, Cachexia, Muscle.

Introduction

Cancer is the second lethal disease in the world. Cancer cells have many typical characteristics that help them to proliferate without host control, and migrate from the original site, establishing in another healthier tissues, producing secondary tumours sites. Therefore, cancer cells need to adapt their metabolism which leads to a higher provision of substrates for their growth and activities, such as increased lactate synthesis, amino acids consumption and decreasing substrate and energy for healthier tissues¹. Most of the cancer cells can release cytokines and factors that promote muscle and adipocyte tissue spoliation leading to a reduced quality of life in patients with cancer ². We analysed whether the tumour culture cells could affect the muscle cells growth or activities when these cells were treated with tumour factors to mimic the same situation as in cancer patient.

Results and Discussion

C₂C₁₂ cells were seeded in 6 wells plates until the confluent state. Then, the experimental protocol distributed these cells into three groups: control (C), C₂C₁₂ cells received DMEM high glucose (as standard culture procedure); C₂C₁₂ cells were treated with 50% DMEM plus 50% 199 medium (199 group) and C₂C₁₂ cells were treated with 50% DMEM plus 50% 199 medium from Walker 256 tumour cells cultivation (W group). The W, 199 and the control groups were observed in 24, 48 and 72 hours. Images were captured and analysed by ImageJ software.

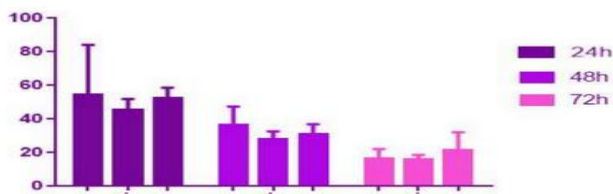


Image 1: Analyses of the confluence layer area of the C₂C₁₂ culture of all experimental groups. C₂C₁₂ cells growth area from control (a), 199 (b) and Walker (c) groups. Images were analyzed by ImageJ software after 24, 48 and 72 h of the experimental procedure.

For these results, we can observe differences among the 24h, 48h and 72h after treating the cells 50% of 199 and 50% of Walker Factor. We observed that there was a decrease in confluence area in both groups 199 and W along the time of analyses, compared to the control group.

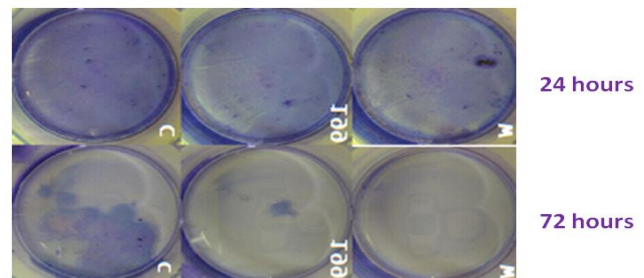


Image 2: Images of the wells from cell culture showing the confluence layer of all experimental groups. 6 wells-plate showing the confluence of Control, 199 medium and Walker factor (W) groups after 24h and 72h of the experiment.

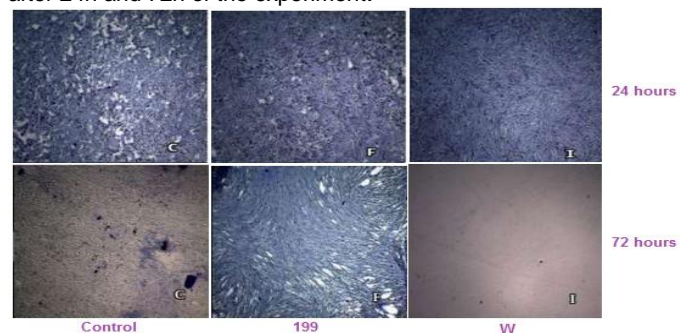


Image 3: Cells morphology of all experimental groups. Legend: Control group (C; C72), 199 (F; F72) and Walker factor (I; I72) in 24 and 72 hours. Magnification 5X light microscope.

Conclusions

There is a correlation between the cellular area and the time of the treatments. Nevertheless, we could observe alterations in cell morphology especially in the W group, suggesting that the tumour cells medium likely contained factors that affected the C₂C₁₂ morphology and activity.

Acknowledgement

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¹ Yano, C. L., Ventrucci, G., Field, W. N., Tisdale, M. J., & Gomes-Marcondes, M. C. C. (2008). Metabolic and morphological alterations induced by proteolysis-inducing factor from Walker tumour-bearing rats in C 2 C 12 myotubes. *BMC cancer*, 8(1), 24.

² Tisdale, M. J. (2010). Cancer cachexia. *Current opinion in gastroenterology*, 26(2), 146-151.