Skeletal Muscle Culture Conditioned Media Influence DNA Synthesis of Tumour Cells

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Abstract

Skeletal muscle culture supernatant contains a large variety of factors that may influence other cell type proliferation. In the present short communication an interesting observation about the influence of muscle culture supernatant on tumour cell proliferation is presented. Myoblast and myofibers culture supernatant modulates tumour cell ^{3}H-TdR incorporation.

Key words: rat skeletal muscle, muscle culture, growth factors, tumour cells.

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Myogenesis is a unique trimodal switch model, in which the myoblast, contrary to the classical model of bimodal switch between cell proliferation and cell differentiation, may also have the option to become a quiescent satellite cell [1]. Therefore, either *in vivo* or *in vitro* myogenesis is a pleiotropic, complicate process in which several factors are involved and their interplay needs further clarification [2]. Three stages can be identified in myogenic line differentiation *in vivo*: determination of a multipotent stem cell to a myoblast, differentiation of the committed myoblast already expressing muscle specific genes to a multinucleate myofiber and myofiber maturation/differentiation during which a variety of myosin isoforms are expressed [3]. *In vitro* two stages are easily recognized: the first is the stage of active myoblasts proliferation, the second is the single myoblasts fusion and the differentiation of myotubes with a critical passage between the two stages from 40 to 56 hours culture time.

In any case, as in a variety of cell types, myoblast proliferation and myofiber differentiation are events mutually exclusive [2] and the molecular basis of their antagonism remains largely unknown. When myoblasts are placed in tissue culture, added serum and exogenous growth factors control their entry into differentiation stage and this repression-type mechanism is probably related to growth factor concentration above and below a critical threshold [4].

In the present short communication we present an observation about the capability of conditioned media from myoblast culture to modulate tumour cell proliferation measured with ^{3}H-TdR incorporation.

Methodology

Skeletal muscle culture: neonatal rat skeletal muscle cells are explanted from leg and cultured according to Cantini *et al.* [5, 6].

Tumour cell cultures: mouse Neuroblastoma N2A and African green monkey Kidney VERO cells were cultured in DMEM medium according to standard methods [7, 8]. Muscle culture supernatant was added at the final concentration of 1, 5, 10, 20, 40% of the normal growing cellular medium. In controls, the same percents of the normal tumour cells culture medium was added.

Cell proliferation: ^{3}H-TdR incorporation was assayed according to Arslan *et al.* [9]. Results of ^{3}H-TdR incorporation were expressed as a percent of ^{3}H-TdR incorporation of the untreated controls.

Results and Discussion

In Fig. 1 the effect of the supernatant of 72 hours of neonatal rat myoblasts on murine N2A and monkey VERO cells is reported. The myoblast conditioned medium was added at different percents from 1 to 40% to the normal growing medium of the cells. In N2A tumour cells, the increase of ^{3}H-TdR incorporation is directly proportional to the increasing percents of the myoblast culture medium. The same myoblast supernatant added in the same percent to VERO cells in culture seems to have no effect on proliferation, showing only a modest increase in ^{3}H-TdR

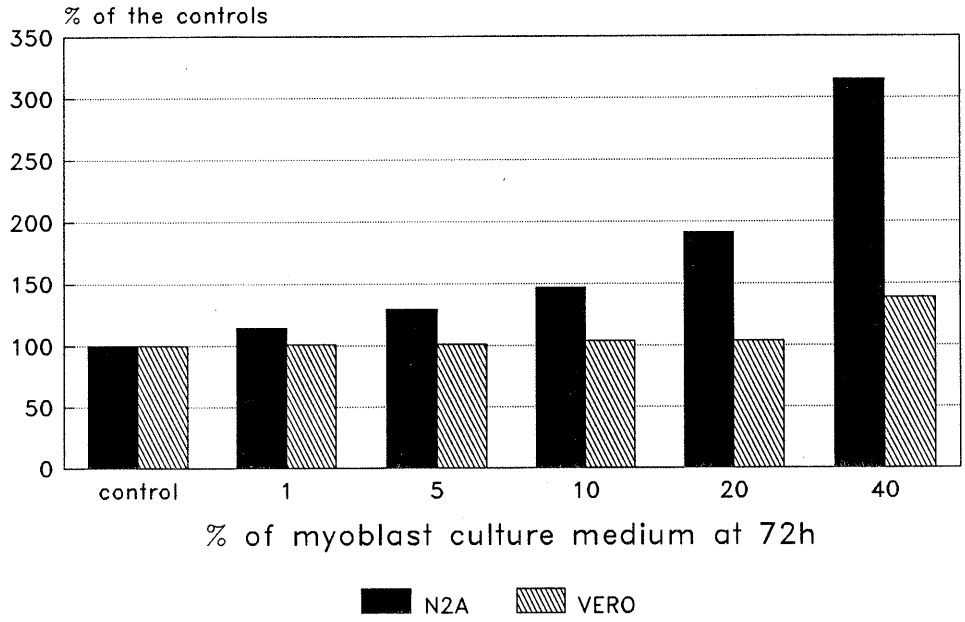

Figure 1. ^{3}*H-TdR incorporation of murine neuroblastoma cells, N2A and monkey VERO tumour cells in the presence and in the absence (controls) of myoblast growth medium. Results are expressed as a percents of the control* ^{3}*H-TdR incorporation (100%).*
Each experimental point is a mean of 6 measurements.
N2A and VERO cells were seeded into DMEM medium added with 10% heat-inactivate FBS and antibiotics at the concentration of 25,000 cells/well. In each well the muscular culture medium was added v/v to final concentration of 1, 5, 10, 20, 40% of normal growing tumour cell medium. Tumour cell cultures were incubated for 48 hours. ^{3}*H-TdR, specific activity was 5Ci/nmol and was pulsed 6 hours before cell harvesting.*
N2A: means of 4 experiments
VERO: means of 3 experiments

incorporation at the concentration of 40% added v/v to the normal growing cell medium. It is interesting to underline that the effect of the myoblast medium on the ^{3}H-TdR incorporation is clear only when the medium is added to the N2A tumour cells at 72 hours of myoblast culture time. At 40 hours of myoblast culture time either the addition of the conditioned medium to the N2A tumour cells is without effect or a slight decrease of ^{3}H-TdR incorporation is observed. In VERO cells the decrease of ^{3}H-TdR incorporation when the supernatant of the 40 hours myoblast culture medium was added, is more evident (data not shown). At 40-56 hours of myoblast culture, myoblasts normally enter into differentiation stage and start to fuse, the concentration of the growth factors responsible to sustain myoblasts proliferation must decline.

Under our experimental conditions, it is impossible to distinguish the cellular origin of the growth factor/s responsible for the observed increase or decrease of ^{3}H-TdR incorporation in tumour cells and why these growth factors seem specific only for certain types of cells.

The data here presented are very preliminary experiments but the increase of ^{3}H-TdR incorporation in N2A cells when 70 hours myoblast culture medium was added and, even more interesting, the decrease of ^{3}H-TdR incorporation in VERO and N2A cells when 40 hours medium was added will prompt us to further investigations.

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