Effect of proline-rich polypeptide on various lines of tumour cells, normal bone marrow and giant-cell tumour stromal tissue

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Introduction. The rapid progress of cell technologies in the recent decades was largely brought about by the discovery of bone marrow stromal stem cells (or multipotent mesenchymal stromal cells, MMSCs) [1] and their wide use in medical practice. Investigations of proline-rich polypeptide (PRP) effect on these cells are of great interest. PRP was first isolated by Galoyan et al. [2] from the neurosecretory granules of N. Supraopticus and N. Paraventricularis of the bovine pituitary, and it can have striking effects on various vital aspects of the living organism as previous studies have shown.

Materials and methods. To obtain human and rat normal bone marrow strains, we prepared a single-cell suspension which was explanted into vials with a complete nutrient medium (4×10⁴ cells/cm²). The cultivation was carried out in a 5 % CO₂ atmosphere at 37 °C. On Day 12–14 when discrete colonies of stromal fibroblasts were formed, the first passage was performed. The cultures were washed with saline and 0.25 % trypsin-treated. Then the cells were counted and transferred into a larger vial (7–8×10³ cells/cm²). Giant-cell tumour (GCT) cells were isolated by trypsinization of minced tumour fragments (1–1.5 mm³) [3]. The cell suspension was explanted into vials with a complete nutrient medium (5×10⁶ cells/cm² per vial 80 cm²). The passage was performed as described above. PRP effect on cell proliferation was studied using strains obtained by passage II–III. 3×10⁴ cells of normal bone marrow were explanted into...
each of 12 vials divided into 4 groups. PRP was added into the vials of each group: Gr. 1 – 1 µg/vial, Gr. 2 – 5 µg, Gr. 3 – 10 µg, and the vials of Gr. 4 were used as controls. The same experiment was used to study PRP effect on GCT stromal cells: 3·10^4 cells of each line were explanted into each of the vials where PRP 5 µg was added.

To study PRP effect on MMSC concentration in the bone marrow, PRP 5 µg (in 0.5 ml of saline) was administered i. m. to rats. On Day 7 or 14 the rats were ether-killed and the tibias were isolated, bone marrow single-cell suspensions were prepared, and 5·10^3 cells were explanted into each of 4 vials (25 cm^2) with a complete nutrient medium. The cultivation was carried out in a 5 % CO_2 atmosphere at 37 °C. On Day 10–12 the cultures were fixed and the grown colonies were counted.

**Results and discussion.** The clonal nature of colonies [4] allowed studying PRP effect on stromal stem cells *in vivo*. A single i. m. injection of PRP 5 µg to rats resulted in a 5–9-fold increase of MMSC concentration in the bone marrow (Fig. 1). These findings are of great importance for cell technologists as they allow significant shortening of the time needed to grow the required number of cells for transplantation. In literature there is no information about any growth factors or other substances which could increase MMSC concentration in the bone marrow after administration into the living organism. Today PRP is the only substance which increases MMSC concentration in the bone marrow over 5-fold when administered i. m. The stromal cells isolated from GCT do not differ in phenotype from MMSCs isolated from the normal bone marrow. Both populations of these cells show a high growth activity after their explantation into a tissue culture. PRP effect on the proliferation of these cells was studied using strains obtained by passage II–III. Vials containing 3·10^4 normal bone marrow cells were divided into 3 groups (3 vials in each group). PRP 0.2 µg/ml of medium was added into each vial in Gr. 1, 1 µg – in Gr. 2, and 2 µg – in Gr. 3. On Day 4 when the cell growth became almost confluent the cultivation was stopped. The results showed that the cell number in the experimental vials increased 1.5-fold as compared to controls, irrespective of PRP concentration. A decreased number of explanted cells (1·10^4) allowed prolongation of the cultivation period to 8 days. Prolonged PRP action on the cells (irrespective of PRP concentration) resulted in a 2-fold increase in the grown cell number as compared to that in 4-days culture (Fig. 2).

To study PRP effect on human GCT stromal cells, 1·10^4 and 3.3·10^4 cells were explanted into vials. The cultivation lasted 8 and 12 days respectively. PRP concentrations in the culture medium were the same. The grown cell number decreased 1.5-fold in the experimental vials as compared to controls (Fig. 2). The longer cultivation time (i. e. the increased length of PRP action on the proliferating cells) did not result in any additional inhibition of growth (Fig. 3). So our findings showed opposite effects of PRP on the stromal cells of normal and tumour tissues.

Inhibition of GCT stromal cell proliferation *in vitro* was the main determinant which guided our studies of PRP effect on other tumour cell lines: Mel. Kor – melanoma (skin cancer), SCOV-3 – ovarian cancer, and two lines of breast cancer – SKBR-3 and MCF-7. PRP 5 µg was added into each vial containing 3·10^5 cells of these lines; the cell proliferation decreased 1.6-fold in the Mel. Kor line and 1.3-fold in the SKBR-3 line. PRP had practically no effect on the other tumour cell lines (Fig. 4).

Numerous groups of researchers are investigating the effect of various growth factors on MMSC proliferation and trying to increase MMSC colony formation by adding such growth factors to cultures. However their findings do not give a clear idea of the effect of certain growth factors on MMSCs [5]. This inconsistency may be accounted for by different methods of cell isolation, different nutrient media and different FBS concentrations in the media, etc. used by different researchers. Standard conditions are extremely important for all investigations of the effect of various growth factors.
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Fig. 3. PRP influences on the effectiveness of colony formation in human GCT cultures: 1 – 1 µg; 2 – 5 µg; 3 – 10 µg; 4 – control

Fig. 4. PRP effect on various tumour cell lines: 1 – control; 2 – 5 µg

Conclusions. PRP 5 µg i. m. administration to rats resulted in a 5–9-fold increase in MMSC concentration in the normal bone marrow. PRP added to cultures of stromal cells of normal bone marrow and GCT had opposite effects on cell proliferation. PRP decreased cell proliferation 1.5-fold in Mel. Kor and SKBR-3 cultures.


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**Для багатого на пролін поліпептиду на різних лініях пухлинних клітин, стромальну тканину нормального кісткового мозку та гіантоклітинних пухлин**

**Резюме**

**Мета** дослідження – вивчення впливу багатого на пролін поліпептиду (ПБП) на ствібову клітину строми кісткового мозку in vivo та in vitro і ліній пухлинних клітин. **Методи.** Виділення стромальних клітин гіантоклітинної пухлини (ГКП) і отримання штамів цих клітин, а також штамів стромальних клітин нормального кісткового мозку, введення ПБП цірум, експлантація в культуральні умови будь-якої мікроультрамікроскопічної клітини, додавання ПБП у культуру клітин. **Результати.** Різні способи і дози введення ПБП цірум збільшують концентрацію мультитипотних мезенхімальних стromальних клітин (MMSC) у кістковому мозку. Додавання ПБП у культури MMSC нормального кісткового мозку призводить до зростання проліфераційної активності клітин у 1,5–2,5 разу, введення ПБП у культури MMSC ГКП інгібіює проліферацію клітин у 1,5–2 разу. У культурних умовах пухлинних клітин спостерігається як пригнічення пухлинних клітин, так і відсутність епітез оновлення на проліферацію. **Висновки.** ПБП цірум підвищує концентрацію MMSC нормального кісткового мозку, а з додаванням ПБП у культурі тканини вибачено його різноманітну дію на проліферацію клітин.

**Ключові слова:** багатий на пролін поліпептид; мультитипотентні мезенхімальні стromальні клітини.

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