ISSN 0233–7657. Biopolymers and Cell. 2011. Vol. 27. N 5. P. 387–389

Disturbances in lipid second messengers generation by stimulated blood lymphocytes in breast cancer

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Aim. *The main objective of this study was the comparative investigation of diverse lipid second messenger (LSM) generation by human peripheral blood lymphocytes (HPBL) at different (5, 10, 30 and 60 s) time points of cell co-stimulation by anti-CD3 and anti-CD28 monoclonal antibodies in norm and breast cancer (BC).* ***Methods***. *Ficoll-Hypaque gradient centrifugation.* ***Results***. *The data obtained indicate that some mechanisms of LSM generation/utilization in stimulated crude HPBL were significantly altered in BC compared to norm. Particularly, the reliable generation of arachidonyl-1,2-diacylglycerol (1,2-DAG) at the initial step (5 s) of cell stimulation observed in norm was depressed in BC and reached the value below the basal level in unstimulated cells. It is important that the disturbances in 1,2-DAG formation in HPBL obtained from patients with BC were identical with those observed earlier in other forms of cancer.* ***Conclusions***. *We conclude that the regularities revealed are common characteristics for all the types of malignancy studied and can be used as additional testing parameters for cancer definition and individual correction of the chemotherapy programs for disease treatment.*

Keywords: blood lymphocytes, lipid second messengers, breast cancer.

Introduction. The establishment of many tumors can occur as a consequence of innate and/or adaptive immune systems escape. Among many factors that are involved in tumor immune escape, the regulatory T cells (T_{reg}) in particular appear to play an important role. It was reported that compared with normal individuals the patients with several types of cancer have an increased prevalence of T_{reg} cells that coexpress CD4, CD25 and Treg marker FOXP3 in the peripheral blood [1, 2], tumor microenvironment and tumor draining lymph nodes. The increase in T_{reg} cells suppressing autoimmunity may also inhibit the immune response against cancer, as evidenced by improved tumor rejection and survival of the tumor-bearing mice that have undergone T_{reg} depletion. However, little has been done to explore the possible relations between the T_{reg} prevalence in the peripheral blood and changes in the lymphocyte signaling machineries. Consequently, it was interesting to investigate the regularities of diverse lipid second messenger (LSM) molecules formation at the early (seconds) time points of peripheral blood lymphocytes costimulation by anti-CD3/CD28 in norm and breast cancer (BC).

Material and methods. 1-[^{14}C]arachidonic acid ([^{14}C]AA, specific activity 58 mCi/mmol) was obtained from «Amersham International» (UK). Chromatographically pure lipid standards were purchased from «Sigma» (USA) and TLC silica gel-60 plates – from

«Merck» (USA). Anti-CD3 (OKT-3) mAb were kindly provided by Dr. A. Altman (La Jolla Institute for Allergy and Immunology, USA). Anti-CD28 mAb were obtained from «BD Bioscience Pharmingen» (USA).

Isolation and stimulation of HPBL. HPBL were isolated from the heparinized whole blood by standard Ficoll-Hypaque gradient centrifugation described by Innes et al. Preliminary labeling of HPBL by [^{14}C]AA TLC was performed as described earlier [3]. Anti-CD3 and anti-CD28 antibodies (2.5 μg) were added to 1 ml of [^{14}C]AA-labeled HPBL suspension (10^8 cells/ml) and incubated for 30 min at 4 °C. After the addition of equal volume of Eagle's medium the excess of unbound anti- bodies was removed by centrifugation (650 *g*, 15 min). After this, resuspended (10^6 cells/ml) HPBL were stimulated by 10 μg/ml IgG at 37 °C in a final volume of 0.5 ml. Reactions were terminated after 5, 10, 30, or 60 s by adding 2 ml of cold chloroform-methanol (1:2, v/v). The cells incubated in the absence of antibodies were used as negative controls.

Lipid analysis. Lipids were extracted according to Bligh and Dyer standard method and fractionated by TLC as described earlier [3]. Lipid fractions were visualized on TLC plates in iodine vapor and identified by comparison with chromatographically pure lipid standards. The distribution of radioactivity in spots was detected by radioscanning (Thin-layer Scanner II LB 2723, Germany). The spots corresponding to different lipid fractions were scrapped into scintillation vials and the amount of radioactivity was estimated using liquid scintillation counter («Roche-Bioelectronique Kontron», France).

Results and discussion. In order to determine the kinetics and nature of LSMs production in activated T cells, we used [^{14}C]AA-prelabeled HPBL, and analyzed the changes in the level of AA-labeled 1,2-diacylglycerol (1,2-DAG), lysophosphatidylcholine (LPC), free AA, monoacylglycerole (MAG) and triacylglycerol (TAG) at the different (5, 10, 30 and 60 s) time points of T cell costimulation by crosslinked anti-CD3/CD28 mAbs.

According to the data obtained at both early (5 s) and relatively sustained (60 s) stages of HPBL stimulation, there are significant differences in the levels of studied LSM molecules in BC compared to norm. It is important that at the initial step (5 s) of cell stimulation the 1,2-DAG level decreased and reached the value below the basal level in unstimulated cells, confirming early depression of phosphoinositide (PI)-cycle as a result of PI-specific phospholipase C inhibition. Notably, the inhibition of 1,2-DAG quick formation was identical with those, observed in ovarian cancer (OC) and three different types of leukemia [3] studied earlier.

At the relatively sustained stage (60 s) following the anti-CD3/CD28 costimulation of HPBL obtained from BC patients, the reliable increase in MAG and TAG levels was observed, correlating with the reduced free AA accumulation. This indicates the possible changes in metabolic pathways realizing fractional interconversions of these lipids. Importantly, the alterations in diverse LSMs generation/utilization dynamic processes in the stimulated crude HPBL, obtained from different patients with BC, display similar regularities but have certain individual characteristics for each patient. The same fact was observed also in OC as well as in two acute forms of leukemia. So, some of disturbances in different LSMs quick formation processes observed in BC are common characteristics for all the types of cancer studied by us.

We conclude that some of disturbances revealed in malignancy can be used as additional testing parameters for cancer definition and individual correction of the chemotherapy programs for disease treatment (Tadevosyan et al., Patents, AM2256, G01N 33/49, 2008; AM 2211, GO1N 33/53, 2009).

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Порушення в утворенні ліпідних вторинних посередників у стимульованих лімфоцитах крові людини при раку молочної залози

Резюме

***Мета**. Основна мета даного дослідження полягала у порівняльному аналізі процесів генерації різних ліпідних вторинних посередників (ЛВП) у загальній масі лімфоцитів периферичної крові людини (ЛПКЛ) на різних етапах (5, 10, 30 и 60 с) костимуляції клітин анти-CD3 і анти-CD28 моноклональними антитілами за норми і при раку молочної залози (РМЗ). **Методи**. Ficoll-Hypaque градієнтне центрифугування. **Результати**. Отримані дані свідчать про наявність значних порушень у механізмах утворення/утилізації ЛВП у стимульованих ЛПКЛ при РМЗ порівняно з нормою. Зокрема, достовірна генерація арахідоніл-1,2-діацил-гліцерину (1,2-ДАГ), що спостерігається за норми, на початковому етапі (5 с) костимуляції клітин виявилася пригніченою при РМЗ та нижчою за контрольний рівень у нестимульованих кліти-*

нах. Треба відмітити, що порушення в утворенні 1,2- ДАГ ЛПКЛ, одержаних від пацієнтів з РМЗ, були ідентичними з виявленими нами раніше при інших формах ракових новоутворень. ***Висновки****. Таким чином, можна зробити висновок, що знайдені закономірності є загальними характеристиками для всіх типів вивчених нами злоякісних пухлин і можуть бути використані як додаткові тест-параметри для виявлення раку та індивідуальної корекції курсу хіміотерапії при лікуванні захворювання.*

Ключові слова: лімфоцити крові, ліпідні вторинні посередники, рак молочної залози.

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Нарушения в образовании липидных вторичных посредников в стимулированных лимфоцитах крови человека при раке молочной железы

Резюме

Цель*. Основной целью данного исследования был сравнительный анализ процессов генерации различных липидных вторичных посредников (ЛВП) в общей массе лимфоцитов периферической крови человека (ЛПКЧ) на различных этапах (5, 10, 30 и 60 с) костимуляции клеток анти-CD3 и анти-CD28 моноклональными антителами в норме и при раке молочной железы (РМЖ).* ***Методы****. Ficoll-Hypaque градиентное центрифугирование.* ***Результаты****. Полученные данные свидетельствуют о наличии значительных нарушений в механизмах образования/утилизации ЛВП в стимулированных ЛПКЧ при РМЖ по сравнению с нормой. В частности, наблюдаемая в норме достоверная генерация арахидонил-1,2-диацилглицерина (1,2-ДАГ) на начальном этапе (5 с) костимуляции клеток была подавленной при РМЖ и находилась ниже контрольного уровня в нестимулированных клетках. Примечательно, что нарушения в образовании 1,2-ДАГ ЛПКЧ, полученных от пациентов с РМЖ, были идентичны с ранее выявленными нами при других формах раковых новообразований.* ***Выводы****. Таким образом, можно заключить, что обнаруженные закономерности являются общими характеристиками для всех типов изученных нами злокачественных опухолей и могут быть использованы в качестве дополнительных тест-параметров для выявления рака и индивидуальной коррекции курса химиотерапии при лечении заболевания.*

Ключевые слова: лимфоциты крови, липидные вторичные посредники, рак молочной железы.

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UDC 577.125
Received 27.07.11