SINGLE LOCAL INTERLEUKIN-2 TREATMENT AGAINST TRANSPLANTED AND SPONTANEOUS MAMMARY CANCER

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Immunotherapy is currently emerging mode of breast cancer therapy as efficacy of traditional therapies seems to reach plateau nowadays. First transplanted generation from non-SPF spontaneous BLRB mammary adenocarcinoma (MAC) were used as appropriate mouse model to examine whether single local interleukin-2 (IL-2) is efficient against mammary cancer. We showed that survival dynamics of syngeneic BLRB males with early emerging transplanted mammary cancer (short subclinical period) taken from naturally arisen female mammary carcinoma could be significantly improved by a single IL-2 treatment ($2{,}5 \times 10^6$ IU per mouse) applied locally two weeks after MAC cell inoculation. However, the same IL-2 therapy mode applied to later emerging tumors (long subclinical period) of the same average size of 5 mm as late as eighth week after tumor cell inoculation notably shortened the survival of tumor-bearing mice.

So, both the fundamental significance and applied implications of biphasic IL-2 effect on mammary cancer growth was shown.

Key words: breast cancer, mouse model, prognostic factors, immunotherapy, interleukin-2.

Abbreviations: BC, breast cancer, in women; MC, mammary cancer, in mice; MAC, mammary adenocarcinoma; interleukin-2, IL-2.

Introduction. Breast cancer affected about each eighth woman in industrial countries; and the incidence of disease is progressively augmented in developing countries.

Numerous studies presented evidence that breast cancer (BC, in women) patients exhibit a T-cell mediated functional immunosuppression, which progresses during tumor growth, so that the early localized disease shows a low-grade defect and advanced disease shows a high-grade generalized immune dysfunction both in mice [1] and BC patients [2, 3]. IL-2 deficiency is essential in this immune impairment. These data provided a rationale of the *in vivo* therapeutic administration of the IL-2 in BC patients [4].

However the therapeutic potential of IL-2 against BC is still not clear both in mice [5, 6] and human patients although distinct therapeutic benefit has been achieved in limited cohorts of patients (discussed in [2]). Two major explanations exist: (i) IL-2 therapy is still applied to BC patients without selection procedure for patients that might benefit from this kind of immunotherapy [7], and (ii) the average tumor growth delay and disease-free and total survival are calculated and compared between the whole treated and untreated groups without distinction between benefit and non-benefit populations. We hypothesized that a therapeutic IL-2 potential might be revealed evaluating therapy efficacy for short and long survivors separately, suggesting that both benefit and non-benefit subgroups exist in IL-2 treated tumor-bearing mouse population. Previously we have shown that promising anti-cancer potential of a single IL-2 treatment

could be revealed in various transplanted mammary carcinoma (MC, in mice) models after therapy efficacy estimation in early and late emerging transplanted mammary carcinoma separately [5].

Materials and methods. We used mice of our own strain of BLRB-Rb (8.17) 1Iem (thereafter called BLRB) with high incidence of naturally arisen mammary adenocarcinoma (MAC) [8]. Animals were maintained in non-SPF, but thoroughly controlled, conditions at Mouse Model Facility of Biothechnology Department of the Institute of Bioorganic Chemistry, Moscow. Mice were fed according Institutional guidance in own modifications and got water *ad libitum.* Each animal had its individual label and was followed through the whole lifespan.

Experimental design. Twenty-six BLRB males were used for MAC cell transplantation. The mice were relatively old (about 12 months of age) to mimic BC appearing in elderly women. MAC cells were taken from naturally arisen female mammary adenocarcinoma. At day $0{,}10^7$ cells from this suspension were injected in male mice s.c. near right fad pads. IL-2 was applied as a single peritumoral IL-2 therapy when the visible tumors were about 5 mm in size (described in [5]). At day 14 seven males with early emerging tumors of about 5 mm in diameter were treated peritumorally with $2{,}5 \times 10^6$ U Chiron IL-2 suspended in 0,5 ml containing 0,9% NaCl and 0,1% Bovine Serum Albumin (BSA). Six control mice with tumors of the similar size were injected in the same manner with 0,5 ml 0,9% NaCl/0,1% BSA at the same time. Six males with late emerging tumors of about 5 mm in diameter were treated by IL-2 in the same manner at day 51; seven control males with the tumors of the same size received control liquid. Then mice were inspected each day for survival and health monitoring. The mean tumor diameter was measured once a week as described in [5].

Statistical Analysis. The different groups consisted of 6—7 animals. The significance of differences in averages was determined by the parametric Student *t*-test. The Mann-Whitney non-parametric *U*-test was used to compare tumor growth kinetics and survival dynamics as was previously described [5].

Results. Early emerging MCs of average size of 5 mm were observed in 13 BLRB males at day 13 after transplantation the MC cell suspension from fast and slowly growing syngeneic female mammary carcinomas. Seven males were treated by a single peritumoral (PT) IL-2 treatment ($2{,}5 \times 10^6$ IU). Six males treated by control liquid constituted a control group. No difference in average tumor diameter was found. However, the IL-2 treated males survived significantly longer ($p < 0{,}05$, *U*-test) (Figure 1, white figures).

The other 13 males had late emerging slowly growing MCs. They reached the average size of about 5 mm at day 51 after MC inoculation. Six males were treated by a single PT IL-2 treatment ($2{,}5 \times 10^6$ IU); seven males treated with the vehicle constituted the control group. The IL-2 treated males survived shorter than the controls. The survival dynamics differed significantly from day 35 until day 135 ($p < 0{,}05$, *U*-test) (Figure 1, black figures).

These data show that a single peritumoral high dose of IL-2 applied to early emerging MACs within second week after tumor cell transplantation prolonged the survival

of tumor-bearing mice. However, delayed IL-2 application (7 weeks after MAC cell inoculation) in the same schedule to late emerging MCs of the same initial average size resulted in reverse effect.

Conclusions. Taken together, these data (i) may demonstrate both benefit and damage of IL-2 therapy of murine mammary carcinoma and (ii) compel to find out prognostic factors that may predict the therapeutic effect of IL-2 therapy. These findings may facilitate to develop basic principles of a selection procedure for BC patients who may benefit from local IL-2 therapy as was proposed by Kedar and Klein. Finally, this task seems to be urgent as, up to now, local IL-2 treatment did not lead to clinically promising outcomes in BC clinic [7].

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ЕДИНСТВЕННЫЙ МЕСТНЫЙ ИНТЕРЛЕЙКИН 2 ПРОТИВ ПЕРЕСАЖЕННОГО И НЕПОСРЕДСТВЕННОГО ГРУДНОГО РАКА

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Иммунотерапия — это способ терапии рака молочной железы, который появился в настоящее время. Первое пересаженное поколение от non-SPF непосредственной грудной аденокарциномы BLRB (MAC) использовалось как соответствующая модель мыши, чтобы исследовать, эффективен ли единственный местный интерлейкин 2 (IL-2) против грудного рака. Мы показали, что динамика выживания syngeneic BLRB мужчины с ранним появлением грудного рака (короткий подклинический период), взятого от естественно возникшей женской грудной карциномы, мог быть значительно улучшен единственной обработкой IL-2 ($2{,}5 \times 10^6$ IU за мышь), примененной в местном масштабе спустя две недели после прививки ячейки MAC. Однако тот же самый способ терапии IL-2 относится к опухолям с более поздним проявлением того же самого среднего размера (5 мм) уже на восьмой неделе после того, как прививка ячейки опухоли сокращала выживание имеющих опухоль мышей.

Так, показано значение и примененные двухфазного эффекта IL-2 на рост рака груди.

Ключевые слова: рак молочной железы, модель мыши, предвещающие факторы, иммунотерапия, интерлейкин 2.