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p-53 expression in cells of chronic inflammatory focus at total low dose-rate γ-irradiation

Експресія р53 у клітинах осередку хронічного запалення при загальному гамма-опромінюванні з малою потужністю дози

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Цель работы: Изучение зависимости экспрессии р53 от дозы в различных типах клеток очага хронического воспаления при общем гамма-облучении с малой мощностью дозы.

Материалы и методы: Исследование выполнено на 96 крысах-самцах линии Вистар. Хроническое воспаление было индуцировано инъекцией раствора каррагенена в предварительно подготовленный подкожный воздушный мешок. Облучение выполнялось к 3-му и 7-му дню воспаления в дозе 0,1, 0,5, 1,0 Гр, мощность дозы 20 мГр/час. Экспрессия р53 оценивалась при помощи иммуногистохимического анализа в макрофагах, лимфоцитах и фибробластах.

Результаты: р53 экспрессируется в воспалительных клетках даже без облучения, возможно, как реакция на повреждение ДНК медиаторами воспаления или для очищения путем апоптоза очага воспаления от клеток, которые выполнили свою функцию. Экспрессия р53 увеличивалась с увеличением дозы гамма-облучения во всех исследованных типах клеток очага воспаления при всех сроках облучения; доза 0,1 Гр не приводила к статистически достоверным изменениям интенсивности экспрессии р53, по сравнению с контролем, для всех типов клеток во все сроки облучения.

Выводы: Полученные в работе низкие значения экспрессии р53 при дозе 0,1 Гр отражают низкий уровень активности супрессии онкогена по отношению к высокой пролиферативной активности и активности перекисного окисления в очаге воспаления при тех же дозах. Таким образом, можно предположить, что онкогенный потенциал хронического воспаления может быть максимально реализован при дозе облучения 0,1 Гр.

Ключевые слова: хроническое воспаление, гамма-облучение с малой мощностью дозы, экспрессия р53.

Purpose: To study dose dependence of p53 expression in various types of inflammatory cells at total low-dose γ-irradiation.

Material and Methods: The study was performed on 96 male Wistar rats. Chronic inflammation was induced by injection of carrageenan solution into previously prepared subcutaneous air pouch. Irradiation was performed by day 3 and 7 of inflammation at doses of 0.1, 0.5, 1.0 Gy, dose-rate 20mGy/h. p53 expression was estimated with immunohistochemical assay in macrophages, lymphocytes, and fibroblasts.

Results: p53 is expressed in the inflammatory cells even without irradiation, probably, as a response to the DNA damage by mediators of inflammation or for cleansing of the inflammatory focus from cells, which completed their function, by apoptosis; p53 expression increased with the dose of γ-radiation in all investigated types of inflammatory cells at all used terms of inflammation; the dose 0.1 Gy did not lead to statistically significant changes of p53 expression intensity in comparison to controls for all cell types at all terms of inflammation.

Conclusion: The low value of p53 expression at dose 0.1 Gy shows obtained in this study low level of oncogen suppression activity in respect of high proliferation and peroxidation activity in the inflammatory focus at the same dose. Thus, it can be supposed, that oncogenous potential of chronic inflammation can be realized to the most at irradiation with dose 0.1 Gy.

Key words: chronic inflammation, low-dose γ-radiation, p53 expression.

Inflammation has long been associated with the increased risk of cancer. This is particularly valid for chronic inflammation, which is characterized by intensified cell proliferation and considered a preneoplastic condition. Over the last decade it has been clearly demonstrated in humans and in animal models that inflammation, especially in its chronic form, significantly contributes to the neoplastic transformation [1, 2]. At the same time a lot of statistical data, concerning radiation carcinogenesis have been accumulated, as well as molecular mechanisms of DNA damage and repair from ionising radiation have been examined by the present moment of time [3–5]. However, little is known about the interaction of low-intensity irradiation and chronic inflammation in mutagenesis. This problem is especially important for Ukraine, Belarus and Russia after Chernobyl accident [3–5].

According to Ames et al. [6], mutations in several critical genes, such as the p53 tumor suppressor gene, can lead to tumors. Damage to the DNA constituting these genes by reactive oxygen species (ROS) may contribute to mutagenicity. More rapidly dividing cells would be most prone to errors in DNA replication and repair, particularly at key regulatory sites (e.g., tumor suppressor DNA regions) [7]. In turn irradiation also leads to DNA damage with the possible following oncogenous transformation. Particularly, it concerns the effect of low-rate (chronic) γ-radiation, which is considered to be the most harmful, and namely this kind of radiation influenced people living on radiation contaminated

territories and produced high rate of radiation-induced neoplasia [3–5].

The aim of this study was to determine the level of p53 expression in the inflammatory cells at low-dose γ-irradiation. It makes possible to estimate cellular response to DNA damage and to foresee the cell fate: either cell division block up to apoptosis or possible oncogenous transformation.

Materials and Methods

The carrageenan-induced air pouch-type granulomatous inflammation served as a model of chronic inflammation. Ninety-six male Wistar rats weighing 180-200 g, were lightly anesthetized with diethyl ether and 12 ml of sterile air were injected SC in the back to make the air pouch oval by shape. Thirty hours later, 2 ml of a 0.5% (weight to volume) solution of carrageenan in PBS were injected into the air pouch at light diethyl ether anaesthesia. The carrageenan solution was sterilized by autoclaving at 121°C for 15 min [8].

For exposing the rats to gamma-radiation gamma-irradiator OB-6 (^{137}Cs, 20 Ci, 14.3 μGy/sec at 1m, Germany) was used. Doses of 0.1, 0.5, and 1.0 Gy were delivered within 4.8, 24, and 48 hours, accordingly. Irradiation was performed at days 3 and 7 of inflammation that corresponds to peaks of macrophage and fibroblast proliferation, accordingly. One part of animals was sacrificed immediately after irradiation to investigate the immediate effect of radiation. Another part was sacrificed some time later after irradiation to investigate the delayed effect of radiation, namely 4 days later for animals irradiated at day 3 of inflammation and 7 days later for those irradiated at day 7 of irradiation.

The animals were sacrificed with diethyl ether. The rats were treated in accordance with the procedures approved by the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, USA, and Public Health Service Policy on Human Care and Use of Laboratory Animals, Office of Laboratory Animal Welfare, National Institutes of Health, USA.

Granuloma tissue was excised with a scalpel and fixed in 10% neutral buffered formalin for 24 h. Fixed tissues were washed in PBS solution, dehydrated through a graded series of ethanol (70-100%), cleared in xylene, embedded in paraffin, and sectioned (5 mcm).

For p53 estimation primary antibody (Dako Mouse Anti-p53 PAb240) and DakoCytamotion EnVision+ Dual Link System-HRP (DAB+) were used [10]. After deparafination and hydration tissue sections were rinsed in PBS and heated at 95 °C in DakoCytamotion Target Retrieval Solution for 30 min. After rinsing in PBS, Dual Endogenous Enzyme Block was applied on slides for 10 minutes to deprive endogenous peroxidase with following rinsing in PDS for 5 min. Then primary antibody at 1:100 dilution was applied with the following incubation for 30 minutes at room temperature. After double rinsing slides in PBS for 5 min each, Peroxidase Labelled Polymer was applied followed by incubation for 30 min at room temperature. After double rinsing slides in PBS for 5 min each, Substrate-chromogen was applied for 10 min followed by rinsing in distilled water, counterstaining with Mayer's hematoxylin for 30 sec and coversliping. Microscopic study was performed with the use of microscope «Olympus» BX51. [p53 positive cells/total cells] x1000 was taken as the p53 index.

Non-paired Student test was used for statistics. A p value less than 0.05 was considered statistically significant. SPSS 10.0 was used for all statistical calculations.

Results and Discussion

In Fig. 1 the dose-dependencies of p53 expression intensity in various types of inflammatory cells are presented for animals, which were irradiated on day 3 of inflammation. Fig. 1a corresponds to immediate radiation effect; Fig. 1b corresponds to delayed radiation effect, where sacrification was performed 4 days later after irradiation completion. As it can be seen from Fig. 1a, p53 expression for immediate radiation effect is observed for all three types of the cells. However, it is more expressed in macrophages and lymphocytes. It is known to occur because day 3 of chronic inflammation in this model corresponds to maximum of macrophage and lymphocyte reaction [9]. p53 expression is significantly increased only at doses 0.5 and 1.0 Gy in comparison to control in all types of inflammatory cells. It is interesting to note that in controls and at a dose of 0.1 Gy p53 expression is completely absent (it can be also explained by low proliferative activity of fibroblast on day 3 of inflammation [9]), while at doses of 0.5 and 1.0 Gy p53 expression increases with dose.

Without irradiation (in the controls) p53 expression is observed in macrophages and lymphocytes at immediate sacrifiction as well as at delayed sacrification. As it is known inflammation itself can result in activation of p53 expression by two mechanisms: 1) as response to DNA damage by mediators of inflammation (e.g. ROS); 2) as a link of apoptosis, stipulated by completion of inflammatory cell functioning in the inflammatory focus. The first mechanism probably determines p53 expression in the controls, which is caused by oxidative explosion in macrophages and can be seen in Fig. 1a. The manifestation of the second mechanism is seen in Fig. 1b for macrophages and lymphocytes in term, corresponding to the ending of their reaction (day 7 of inflammation). At irradiation p53 expression is significantly increased with dose at day 3 of inflammation for both immediate and delayed cases only at doses of 0.5 and 1.0 Gy. As for fibroblasts, on day 7 of inflammation (Fig. 1b), when their reaction reaches maximum, p53 expression is observed at all doses as well as in the controls and it increases with the

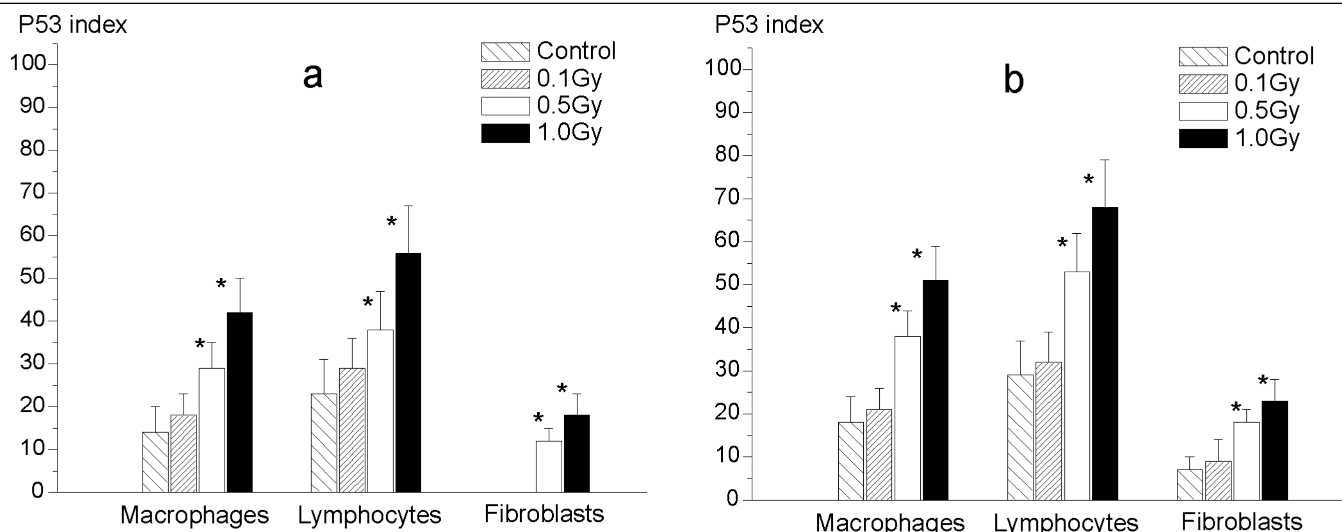

Fig. 1. γ-radiation dose dependence of p53 index in the inflammatory cells. Irradiation was performed on day 3 of inflammation induction. a — immediately after irradiation; b — day 7 of inflammation induction (day 4 of irradiation). Asteriscs indicate statistical differens with control

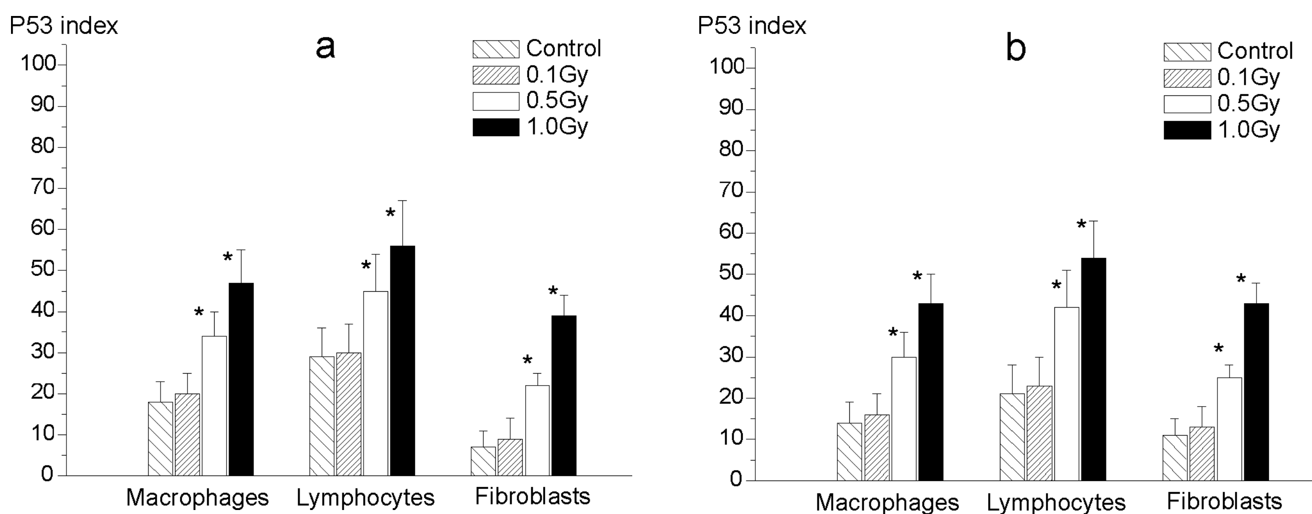

Fig. 2. γ-radiation dose dependence of p53 index in the inflammatory cells. Irradiation was performed on day 7 after inflammation induction. a — immediately after irradiation; b — day 14 after inflammation induction (day 7 after irradiation)

dose, however, the increase at dose 0.1 Gy in comparison with control is not statistically significant as for the other cell types.

In Fig. 2 the dose-dependence of p53 expression is presented for animals, which were irradiated on day 7 of inflammation. Fig. 2a corresponds to immediate radiation effect; Fig. 2b corresponds to delayed radiation effect, where sacrification was performed 7 days after irradiation completion (day 14 of inflammation). In both cases p53 expression is increased with the dose for all types of the inflammatory cells. As before, the dose 0.1 Gy does not influence p53 expression for all the cell types at all terms of inflammation. For higher doses (0.5 and 1.0 Gy) positive dose-dependence is observed. As in previous cases, lymphocytes production p53 was highest. It should be emphasized that high values of p53 expression in the controls are probably connected with inflammatory focus cleansing from macrophages and lymphocytes on day 7 (Fig. 2a) and from all inflammatory cells on day 14 of inflammation (Fig. 2b).

Thus, the following conclusions can be drawn:

p53 can be expressed in the inflammatory cells even without irradiation, as a response to the DNA damage by mediators of inflammation or for cleansing of the inflammatory focus from cells, which completed their function by apoptosis. The intensity of p53 expression depends on the type of the cells and on the terms of inflammation;

p53 expression increases with the dose of γ-radiation in all types of inflammatory cells at all terms of inflammation;

the lowest dose (0.1 Gy) does not produce to statistically significant changes of p53 expression intensity in comparison to control for all cell types at all terms of inflammation.

According to previous data [11] radiation dose 0.1 Gy increases proliferation of fibroblasts and macrophages more then higher doses (0.5 Gy and 1.0 Gy), making them more liable to radiation mutation. At the same time che miluminescence assay revealed high activity of lipid peroxidation processes at 0.1 Gy, especially at irradiation on day 7 of inflammation, which may point to high probability of DNA damage [12].

Conclusion

Obtained in this study low value of p53 expression at a dose of 0.1 Gy shows low level of oncogen suppression activity in respect of high proliferation and peroxidation activity in the inflammatory focus at the same dose. Thus, it can be supposed, that oncogenous potential of chronic inflammation can be fulfilled to the most at exposure to a dose of 0.1 Gy.

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