RESEARCH ARTICLE

Peculiarities of neoplasms appeared after total body irradiation and homeostasis parameters in rats [version 1; peer review: awaiting peer review]

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Abstract

Background: Tissue damage and disruption of metabolic processes as a result of total body irradiation (TBI) could lead to tumorigenesis.

Methods: Female rats (25 of 32) were X-irradiated with a 6-Gy dose. On month 12±1 animals were sacrificed. The alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, lactate dehydrogenase (LDH), Ca2+, creatinine, glucose, phosphorus, urea, uric acid, total protein, pO2, pCO2, pH, and blood cell count were evaluated in blood. Tumors were examined histologically.

Results: On 12±1 months after TBI, 76% of rats had visually detected tumors, histologically classified as benign fibro adenomas. Metabolic, hematological changes versus healthy control indicated disturbances in the homeostasis system. The blood lactate level was typically higher in animals with tumors than without. The ratio of tumor weight to lactate (or LDH) level in blood was 1±0.5 in the 63% of rats and histological analysis revealed the signs of biphasic hyperplasia of glandular lobes and connective tissue stroma, associated with secretory and proliferative activities in tumor. In animals with high values of this ratio (≥1.5) neoplasms were represented by fibrous and glandular tissues presenting a predominant stromal fibrous component, associated with the prevalence of high proliferation in tumor. While in 20% of tumor bearing rats with the low ratio (<0.5) predominate an epithelial structure with homogeneous basophilic content in the glandular lumens, suggesting the domination of
secretory activity in tumor.

**Conclusions:** TBI promoted the alterations of hematological and biochemical parameters of homeostasis in rats and provoked the appearance of benign tumors one year after. The ratio of tumor mass to lactate (or LDH) level in blood seems to be an informative indicator of the histological particularities of tumors, suggesting the prevalence of proliferative or secretory activity, or the balance between them.

**Keywords**
rat, total body irradiation, tumorigenesis, ratio of tumor mass to lactate, LDH

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**Author roles:** Snezhkova E: Conceptualization, Investigation, Writing – Original Draft Preparation; Voronina O: Conceptualization, Investigation; Zadvornyi T: Investigation; Todor I: Data Curation, Investigation; Lukianova N: Conceptualization, Data Curation; Melnyk V: Investigation, Methodology; Sakhno L: Investigation, Writing – Review & Editing; Bardakhivska K: Investigation, Methodology; Chekhun V: Conceptualization, Methodology, Supervision; Nikolaev V: Conceptualization, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

**Grant information:** This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 734641 (Nanoporous and Nanostructured Materials for Medical Applications [NanoMed]).

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**How to cite this article:** Snezhkova E, Voronina O, Zadvornyi T et al. **Peculiarities of neoplasms appeared after total body irradiation and homeostasis parameters in rats [version 1; peer review: awaiting peer review]** Open Research Europe 2022, 2:95 https://doi.org/10.12688/openreseurope.14515.1

**First published:** 10 Aug 2022, 2:95 https://doi.org/10.12688/openreseurope.14515.1
Introduction

The risk of neoplasm appearance after total body irradiation (TBI) in moderate doses has been demonstrated in numerous experimental investigations as well as in the clinical studies of Japanese atomic bomb survivors and of the consequences of the Chernobyl accident. Tumors have also been reported in patients undergoing radiation treatment and diagnostics. The appearance of tumors after irradiation is often related to the fact that the radiation can lead to life-threatening multiple organ failure, dysfunction syndrome and inflammation. After TBI, a variety of diseases frequently develop, such as extrarenal bile obstruction, intrahepatic cholestasis, infiltrative liver disease, which is accompanied by increased serum alkaline phosphatase (ALP), as well as pancreatitis with an elevated level of amylase. The most classically recognized symptom of acute radiation sickness is hematopoietic syndrome resulting in reduced count of platelet, leukocytes, and erythrocytes. Even low levels of exposure can lead to bone marrow failure, potentially lethal hemorrhage, or infections. Decreased partial arterial oxygen pressure and an increased level of carboxyl groups are well known signs of the pathological states in acute radiation syndrome and tumorogenesis.

Tumor hypoxia leads to therapeutic resistance and promotes aggressive tumor behavior and metastases. Tumor clones dramatically alter their metabolic activity to meet the metabolic demands of relentless cell division. The highly conserved metabolic pathway of fermentative glycolysis is exploited by rapidly growing tissues and tumors. Lactate generation is a cellular process necessary for maintaining glycolytic flux and facilitating the removal of pyruvate from the cell. The interconversion of pyruvate to lactate is mediated by lactate dehydrogenase (LDH) and results in the oxidation of NADH to NAD+. The decrease in absorbance due to the consumption of NADH was measured at 340nm and is proportional to the ALT activity (LD) catalyzed reaction with NADH to produce lactate and α-oxoglutarate to form pyruvate and glutamate. The pyruvate enters a lactate dehydrogenase (LD) catalyzed reaction with NADH to produce lactate and NAD+. The shift in absorbance due to the consumption of NADH was measured at 340nm and is proportional to the ALT.

In the present study we aimed to investigate the main hematological and biochemical homeostasis parameters and peculiarities of tumorigenesis in rats after total body irradiation in sub-lethal doses.

Methods

Animal model

Two weeks before the start of the study (approved by the IEPOR Committee on Bioethics, protocol number 4 dated 16 April 2015 for experiments with rats in Nanomed project), 32 white, random bred, female rats weighing ±140g from IEPOR animal house, were taken for an adaptation period and had ad libitum access to standard food and water, in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). All efforts were made to ameliorate any suffering of animals: rats had ad libitum access to standard food and water during experiment and they were sacrificed under total anesthesia. At the beginning of the experiment all animals were randomly allocated into groups: 1—irradiated rats (n = 25); 2—healthy rats (n = 7), weighed and examined every month for visual tumor appearance. On month 12±1 most irradiated animals had visually detectable tumors. This period was selected as optimal for animal sacrifice because of the risk of development of tumor necrosis, which is unfavorable for histological study. After dissection of all 32 animals, tumors were found in 19 rats after irradiation; this group was named Ir&Tum (n=19); the group of irradiated animal without tumor was named Ir (n=6) and healthy rats, were the control (n=7).

Irradiation

Ionizing radiation was delivered to eight-week-old rats weighing 194 g ± 18 (prior to irradiation), using an X-ray RUM-17 irradiator with a working current of 10 mA, 0.5 mm Cu filter, 30 cm-target distance in a rotating box with four separate sections (24 × 15 cm) for four rats; exposure time was 11 min. Control group animals underwent the same procedure, excluding irradiation. The absorbed sublethal dosage per rat was 6 Gy (54.5 cGy/min during 11 min), which was applied in the morning between 10–11 a.m.

Blood collection for blood cell count, biochemical parameters, lactate, pO2, pCO2, pH

Terminal citrate blood samples and heparin blood samples (for lactate, pO2, pCO2, pH) were obtained via puncture of abdominal vein.

Blood cell count

Blood cell counts were performed using a light microscope.

Lactate, pO2, pCO2, pH level in blood

Lactate, pO2, pCO2, pH levels were measured using an ABL800-Flex gas analyzer (Radiometer, Denmark, Copenhagen).

Blood biochemical parameters

All biochemical parameters of serum were measured using reagent kits from Beckman Coulter and an autoanalyzer (Beckman Coulter AU-480, USA). Manufacturer’s instructions were followed.

The alkaline phosphatase (ALP) level was determined by measuring the rate of conversion of p-nitro-phenylphosphate (pNPP) in the presence of 2-amino-2-methyl-1-propanol (AMP) at pH 10.4. Alanine aminotransferase (ALT) transfers the amino group from alanine to α-oxoglutarate to form pyruvate and glutamate. The pyruvate enters a lactate dehydrogenase (LD) catalyzed reaction with NADH to produce lactate and NAD+. The decrease in absorbance due to the consumption of NADH was measured at 340nm and is proportional to the ALT.
activity in the sample$^{22}$, aspartate aminotransferase (AST) catalyzes the transamination of aspartate and $\alpha$-oxoglutarate, forming L-glutamate and oxalacetate. The oxalacetate is then reduced to L-malate by malate dehydrogenase, while NADH is simultaneously converted to NAD$^+$. The decrease in absorbance due to the consumption of NADH was measured at 340nm and is proportional to the AST activity in the sample$^{22}$; amylase was measured using 2-chloro-4-nitrophenyl maltotrioside as substrate$^{23}$, lactate dehydrogenase (LDH): lactate and NAD$^+$ are converted to pyruvate and NADH catalyzed by LD. NADH strongly absorbs light at 340nm, whereas NAD does not. The rate of change of absorbance at 340nm is directly proportional to the LD activity in the sample$^{23}$; Ca$^{2+}$ is based on calcium ions (Ca$^{2+}$ ) reacting with Arsenazo III (2,2’-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]-bisbenzenear-sonic acid) to form an intense purple-colored complex$^{25}$; creatinine reacts with picric acid at alkaline pH to form a yellow-orange complex. The rate of change in absorbance at 520/800nm is proportional to the creatinine concentration in the sample$^{26}$; glucose$^{27}$ is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G6P-DH) specifically oxidizes G-6-P to 6-phosphogluconate with the concurrent reduction of nicotinamide adenine dinucleotide (NAD$^+$) to nicotinamide adenine dinucleotide, reduced (NADH). The change in absorbance at 340/380nm is proportional to the amount of glucose present in the sample$^{28}$; phosphorus measurement was based on a modification of the method$^{28}$. The absorbance at 340/380nm is directly proportional to the inorganic phosphorus level in the sample$^{29}$; urea is hydrolyzed enzymatically by urease to yield ammonia and carbon dioxide. The ammonia and $\alpha$-oxoglutarate are converted to glutamate in a reaction catalyzed by L-glutamate dehydrogenase (GLDH). Simultaneously, a molar equivalent of reduced NADH is oxidized. Two molecules of NAD$^+$ are oxidized for each molecule of urea hydrolyzed. The rate of change in absorbance at 340nm, due to the disappearance of NAD$^+$, is directly proportional to the urea concentration in the sample$^{29}$; uric acid is determined by measurement of hydrogen peroxide produced by the uricase reaction$^{30}$. Total protein was measured by the biuret method: cupric ions in an alkaline solution react with proteins and polypeptides containing at least two peptide bonds to produce a violet-colored complex. The absorbance of the complex at 540/660nm is directly proportional to the concentration of protein in the sample.

**Histology of tumor tissues**

Tumor tissues were carefully isolated for histological examination, weighed, measured and fixed in 4% neutral buffered formalin. Fixed tissues were dehydrated and embedded in paraffin, and then cut into serial 4µm-thick sections. The histopathological characteristics were obtained on tumor tissue sections after hematoxylin & eosin staining in 19 rats with tumors.

**Statistical analysis**

Mean values and standard deviations were calculated. Differences between groups were evaluated statistically with Fisher’s test followed by a Student’s t-test for independent samples. Group differences were considered significant when $p < 0.05$. For biochemical and hematological parameters, statistical analysis of the results was carried out using Mann-Whitney U nonparametric comparison. Statistical significance was set at $p < 0.05$.

**Results**

Twelve months after irradiation in 76 % of females (19 from 25, group- Ir&tum) tumors were detected visually in the lower part of the animal’s body associated with mammalian glands. Histological analysis indicates that all the tumors found were fibro of the breast (Figure 1–Figure 3). Fibroadenoma is a benign neoplasm, in which the lobular structure of the gland is preserved. But the proliferation of both glandular and stromal elements is clearly manifested, atypia is not observed in any of the components. Glandular and stromal components of the gland are clearly visible on histological preparations of tumors. The glandular parenchyma is represented by secretory...
(tubulo-alveolar) units and their clusters with the layers between formed by fibrous connective tissue.

No tumor was found in any control.

The statistical increase of alkaline phosphatase was observed and compared with the control group. Amylase activity in the plasma of rats in both irradiated groups was statistically higher one year after irradiation than in the control (Table 1).

Hematological parameters in major cases demonstrate a worsening in both irradiated groups. White blood cells and platelet levels were found to be significantly lower in the blood of irradiated animals with a tumor in comparison with healthy control as well as irradiated animals without tumors (Table 2).

The lactate level was significantly elevated in the tumor group compared to the control. In both irradiated groups pO₂ was significantly reduced, and in the irradiated group without tumor pCO₂ increased, as opposed to the control group (Table 3).

The first tumor was visually detected five months post-irradiation (Table 4) but the majority of tumors appeared later, in the tenth (n=9) and thirteenth (n=5) month after irradiation (Table 4).
Table 1. Alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), Ca$^{2+}$, creatinine, glucose, phosphorus, urea, total protein, and uric acid levels in blood plasma of rat groups: healthy animals (control), irradiated (Ir), with tumor detected visually one year after total body irradiation (Ir&Tum).

| Rat group   | ALP, U/L | ALT, U/L | Amylase, U/L | AST, U/L | LDH, U/L | Ca$^{2+}$ mmol/L | Creatinine, µmol/L | Glucose, mmol/L | Phosphorus, mmol/L | Total protein, g/L | Urea, mmol/L | Uric Acid, µmol/L |
|-------------|----------|----------|--------------|----------|----------|-----------------|-------------------|----------------|-------------------|------------------|-------------|-----------------|
| Control     | 111±33   | 54±17    | 1701±251     | 183±20   | 935±317  | 2.2±0.1         | 41±8.6           | 3.9±0.9         | 2.3±0.7           | 63±8             | 6.6±1.1     | 72±9            |
| Ir          | 250±40*  | 66±11    | 2609±380*    | 181±33   | 600±59   | 2.4±0.1         | 34±2              | 5.3±0.4         | 1.5±0.1           | 63±3.1           | 5.6±1       | 60±5            |
| Ir&Tum      | 151±77   | 57±29    | 2180±679*    | 171±84   | 768±336  | 2.0±0.4         | 32±5.5           | 4.0±1.1         | 1.3±0.4           | 55±10.4          | 5.6±1.1     | 78±46           |

*According to the Mann-Whitney U test the result is significant at p < 0.05 (in comparison with control)

± Standard deviation

Table 2. Hematological parameters of rats (control: healthy animals, irradiated: Ir, with visually detected tumor after irradiation: Ir&Tum): hemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), stab and segmented neutrophiles, lymphocytes, monocytes, and platelets.

| Rat group   | Hb, g/L  | WBC, 10^9/L | RBC, 10^12/L | Eosinophils, 10^9/L | Neutrophiles, stab., 10^9/L | Neutrophiles, segmented, 10^9/L | Lymphocytes, 10^9/L | Monocytes, 10^9/L | Platelets, 10^10/L |
|-------------|----------|-------------|--------------|-------------------|-----------------------------|-------------------------------|--------------------|------------------|-------------------|
| Control, n=7| 131±12.4 | 10±1.1      | 5±0.9        | 0.04±0.04         | 0.08±0.03                   | 2±0.5                        | 7±1.6             | 0.5±0.2          | 267±29           |
| Ir, n=6     | 132±1.2  | 9±1.6       | 5±0.2        | 0.04±0.04         | 0.0±0.0                     | 2±0.2                        | 7±1.5             | 0.2±0.1          | 304±11           |
| Ir&Tum, n=17| 125±13   | 5.9±1.7*#   | 4.5±0.5      | 0.02±0.04         | 0.1±0.03                    | 1.2±0.5                      | 4.3±1.1           | 0.2±0.2          | 204±57*#         |

*According to the Mann-Whitney U test the result is significant at p < 0.05 (in comparison with control).

≠ According to Mann-Whitney test the result is significant at p < 0.05 (in comparison with Ir)

± Standard deviation

The certain correlation of tumor weight (but not tumor volume, measured manually) with lactate and LDH values in the blood of irradiated animals is shown in Table 4. The animals were distributed in three groupings named K1-K3, according to the coefficient C1 reflecting the ratio of tumor weight to blood lactate level. In the majority of animals (63.2%, n=12, grouping K2) tumor weight was direct related to blood lactate and LDH levels and the C1 coefficient was approximately equal to 1 (0.5<C1<1.5). In the remaining 36.8% of animals, the lactate and LDH levels were not directly related to tumor weight. In the K1 (n=4) grouping, C1 had a value ≤ 0.5, and in the K3 grouping, C1 ≥ 1.5 (n=3, 15.8% of animals). The C1 coefficient correlated (except for rat number 5, with a C1 value of 0.6 on the border of K2 and K1 values) with the C2 coefficient (ratio of tumor weight to LDH level), which confirms the correct distribution of animals in these conditional K groupings and the possibility to use blood lactate or LDH levels for classification.
We investigated the histological specificities of neoplasms in 19 rats from the Ir&Tum group, classified and grouped conditionally in the groupings K1, K2, K3, according to the values of the C1 coefficient.

Neoplasms in the K1 grouping (n=4) with a relatively low tumor weight ratio to lactate level in blood (C1 ≤ 0.5) produced tumors comprised of fibrous and glandular tissues with a predominant epithelial component of a clear lobular structure breast (Figure 1A,B). Glandular structures were formed by a two-layer epithelium without signs of atypia. The inner glandular layer consisted of cuboidal cells with normochromic nuclei. Benign processes were recognized by the presence of myofibroblasts. Basophilic content was noticeable in the lumen of many glands and duct and the cystic extension of the ducts could be observed (Figure 1B). The stroma was homogeneous, hypo-vascular, with signs of fibrosis. The fusiform fibroblasts with elongated nuclei were seen between collagen fibers. No signs of inflammation and hemorrhages in the tumor were observed in the connective tissue stroma.

**Table 3.** Lactate, pO$_2$, pCO$_2$, and pH in the blood by rat group: healthy animals (control), irradiated (Ir), with tumor appearance one year after total body irradiation (Ir&Tum).

| Rat's group    | Lactate, mmol/L | pO$_2$, mm Hg | pCO$_2$, mm Hg | pH    |
|----------------|----------------|---------------|----------------|-------|
| Control, (n=7) | 5.3±0.8        | 60.7±2.3      | 33.8±8.9       | 7.3±0.05 |
| Ir, (n=6)      | 6.3±1.2        | 45.6±3.7*     | 42.6±1.7*      | 7.3±0.05 |
| Ir&Tum, (n=19) | 6.6±1.9*       | 41.1±6.2*     | 38.3±5.9       | 7.3±0.1  |

*According to Student's t-test the result is significant at p < 0.05 (in comparison with control)

**Table 4.** Weight and volume of tumors in the Ir&Tum rat group (n=19) and month of visual detection after total body irradiation; blood lactate dehydrogenase (LDH) and lactate levels; C1: coefficient = Tumor weight/ Lactate level; C2: coefficient = Tumor weight/ LDH *1000. K 1-3: groupings of animals, where: K1 is the grouping of rats with C1 ≤ 0.5; K2: with 0.5 < C1 < 1.5; K3: with C1 ≤ 1.5.

| Rat number | Month of tumor detection after irradiation | Tumor volume, cm$^3$ | Tumor weight, g | LDH, U/L | Lactate level, mmol/L | C1 | C2 | K groupings |
|------------|-------------------------------------------|----------------------|----------------|-----------|-----------------------|----|----|-------------|
| 1          | 13                                        | 0.88                 | 2.28           | 1307      | 5.7                   | 0.4 | 1.7 | K1          |
| 2          | 10                                        | 1.05                 | 1.8            | 668       | 4.2                   | 0.4 | 2.7 | K1          |
| 3          | 11                                        | 3.3                  | 4.24           | 1338      | 8.7                   | 0.5 | 3.2 | K1          |
| 4          | 10                                        | 4.25                 | 4.61           | 1370      | 9.4                   | 0.5 | 3.4 | K1          |
| 5          | 5                                         | 2.68                 | 2.76           | 1401      | 4.5                   | 0.6 | 2.0 | K2          |
| 6          | 10                                        | 4.5                  | 6.66           | 546       | 8.1                   | 0.8 | 12.2 | K2          |
| 7          | 13                                        | 8.77                 | 7.7            | 720       | 8.9                   | 0.9 | 10.7 | K2          |
| 8          | 9                                         | 6.21                 | 7.82           | 621       | 8.5                   | 0.9 | 12.6 | K2          |
| 9          | 10                                        | 1.98                 | 3.41           | 480       | 3.7                   | 0.9 | 7.1  | K2          |
| 10         | 10                                        | 6.63                 | 5.62           | 484       | 5.9                   | 1.0 | 11.6 | K2          |
| 11         | 13                                        | 1.98                 | 4.76           | 735       | 4.8                   | 1.0 | 6.5  | K2          |
| 12         | 10                                        | 4.35                 | 8.32           | 590       | 8.1                   | 1.0 | 14.1 | K2          |
| 13         | 10                                        | 2.77                 | 4.75           | 536       | 4.5                   | 1.1 | 8.9  | K2          |
| 14         | 13                                        | 10.58                | 6.85           | 597       | 5.3                   | 1.3 | 11.5 | K2          |
| 15         | 13                                        | 6.84                 | 8.2            | 826       | 6.1                   | 1.3 | 9.9   | K2          |
| 16         | 10                                        | 7.6                  | 8.71           | 991       | 6.3                   | 1.4 | 8.8   | K2          |
| 17         | 8                                         | 1.79                 | 6.63           | 453       | 4.3                   | 1.5 | 14.6 | K3          |
| 18         | 10                                        | 26.8                 | 19.46          | 659       | 9.6                   | 2.0 | 29.5 | K3          |
| 19         | 8                                         | 25.3                 | 36.8           | 279       | 8.0                   | 4.6 | 131.9 | K3          |
Histological examination of tumors in rats in conditional grouping K2 (n=12) with a tumor weight to blood lactate level ratio $0.5 < C_1 < 1.5$ demonstrated signs of biphasic hyperplasia of the glandular lobes and connective tissue stroma (Figure 2A, B). Neoplasms were represented by hyperplastic breast tissue with a clear lobular structure. Acini were lined with epithelial cells with vacuolated cytoplasm, indicating active synthesis as well as secretion\textsuperscript{31,32}. Epitheliocytes formed a multilayered epithelium, not typical to the glands but without signs of atypia. Nuclei were of typical size with eu- and hetero- chromatin. Mitotic figures were not found. In most animals in this group, small cystic cavities filled with basophilic homogeneous mass (Figure 2B) were observed. The stroma was represented by well-developed collagen fibers, with clearly visible nuclei of the fibroblasts between them. The blood vessels in the tumor were dilated and filled with blood cells. Outside vessels, erythrocytes were visible in a stroma as well as in a parenchyma (Figure 2B).

The parenchyma of tumors in rats from the K3 conditional grouping (n=3) with relatively high values of the ratio of the tumor weight to blood lactate level ($C_1 \geq 1.5$) was shown by a complex, branched tubular-alveolar system, separated from each other by stromal connective tissue. Moreover, the stromal fibrous component predominated in the slides (Figure 3A, B), with clearly visible, well-developed fibers and elongated fibroblasts.

In the K1, K2 and K3 conditional groupings of Ir&Tum rats, the histological features of neoplasms were generally similar, but had some specific differences. In all groups, the tumors were identified as benign (non-cancerous) breast fibroadenomas and signs of proliferation of epithelial and stromal elements.

In the K3 grouping, with maximal values of ratio of tumor weight to lactate ($C_1 \geq 1.5$) the stroma connective tissue was associated with increased proliferation. In the K1 grouping with minimal $C_1 \leq 0.5$, tumor tissue dominated well-developed ductal epithelium with lobular hyperplasia, indicating high secretory activity. In K1 as well as in most K2 rats, a homogeneous basophilic content in the lobule was found. This indicates the high secretory activity of epithelial cells\textsuperscript{31,33}. Signs of inflammation were found only in some animals in the K2 and K3 groupings. In several K2 rats, histological signs of extensive vascular blood supply in tumors were discovered.

**Discussion**

Within five to 13 months after TBI, 76% of irradiated female rats (Table 4) were found to have benign tumors: fibro adenomas, associated with mammalian glands (Figure 1–Figure 3). The peak of tumors appearance (79%) occurred 10–13 months after irradiation. No tumor was detected visually in the controls. We only detected and histologically confirmed benign tumors in our experiment, while Bespalov and colleagues\textsuperscript{1} reported about 40% of malignant tumors from a total number of radiation-induced tumors (80% of animals) 16 months after total body gamma-ray irradiation in doses of 4 Gy (1.34 Gy per min).

Perhaps this is due to the different doses and sources of rat irradiation.

Benign as well as malignant tumors progress, and this process is associated with high energetic demands. Lactate and LDH, the main actors of fermentative glycolysis, meet the energy demands of tumors. Elevated lactate and LDH levels in presence of tumor was described in numerous studies\textsuperscript{15–18,34,35}. Furthermore, increased blood LDH and lactate values were observed in both malignant\textsuperscript{36} and benign\textsuperscript{34,35} growths. In our blood samples, lactate levels in the group of animals with tumors (Ir&Tum) was statistically higher than in healthy animals (Table 3). However, the lactate dehydrogenase activity in the same group of animals was not statistically different, possibly due to high values of the standard deviation (Table 1).

We tried to find a correlation between the ratio of the mass of tumors to lactate/LDH levels in the blood and the histological peculiarities of tumor tissue. For this, the 19 animals with irradiation-induced tumors were divided into three conditional groupings (K1-K3) according to the value of tumor weight ratio to lactate level ($C_1$) (Table 4). We considered it important that the $C_2$ coefficient, which reflects the ratio of tumor weight to blood LDH levels (one of the main actors of lactate metabolism\textsuperscript{31}), correlated with the $C_1$ coefficient, i.e., the ratio of tumor weight to lactate level. This means that the same animals could be divided into the three conditional K1-K3 groupings depending on the values of tumor weight ratio to either lactate or blood LDH levels.

In the majority of animals with a tumor in the conditional K2 grouping (63.20%, n=12), with a value of tumor weight ratio to blood lactate level ($0.5 < C_1 < 1.5$), histological examination revealed signs of biphasic hyperplasia of the glandular lobes and connective tissue stroma (Figure 2 A, B); this indicates secretory and proliferative activities of tumor tissues\textsuperscript{31,32}. Neoplasms in the K3 grouping (Table 4) with relatively high values of tumor weight ratio to lactate level ($C_1 \geq 1.5$, n=3) are represented by fibrous and glandular tissues with domination of the connective tissue stromal component (Figure 3A, -B). This shows the prevalence of proliferative activity of tumor cells\textsuperscript{12,37}. In the K1 grouping with a relatively low ($C_1 \leq 0.5$) value of the tumor weight ratio to blood lactate or LDH levels (Figure 1), the epithelial structure with homogeneous basophilic content dominated the glandular lumens. This is associated with prevalence of high secretory activity of tumor\textsuperscript{31,33}. Thus, the values of the ratio of tumor weight to blood lactate or LDH levels ($C_1$ and $C_2$ coefficients) correlated with histological specificity of tumor tissues. This makes it possible to indirectly evaluate the specificities of the tumor state, indicating the prevalence of proliferative activity, as in the K3 grouping, or secretory one, as in the K1 grouping, or a balance between proliferative and secretory activities of tumor cells (K2 grouping).

The metabolic and hematological unbalances that arose after irradiation\textsuperscript{16} confirms the violation of homeostasis that conducts to tumorigenesis. In the group of irradiated rats without...
tumors, a significant increase in serum alkaline phosphatase (ALP) indicates a metabolic disorder\(^6\), which disrupts homeostasis (Table 1). Markedly elevated ALP is seen predominantly with a number of specific pathologies, such as malignant biliary obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, hepatic lymphoma and sarcoidosis\(^6\).

Amylase activity is depicted in Table 1. A significant increase in the activity of serum amylase (although only 1.5-1.3-fold) was evident in both rat groups one year after irradiation. Increased activity of amylase and lipase is usually a diagnostic sign of pancreatitis\(^6\); these enzymes are released from the pancreas into circulation early in the inflammation process. Blood leucocytes and platelets counts (Table 2) were statistically lower in the Ir&Tum group, indicating and confirming an inhibitory effect of the tumor\(^1\) on hematopoiesis. The statistically significant drop of pO\(_2\) was found in both groups of irradiated animals (Table 3), which indicates the presence of pathological processes\(^1\).\(^12\). Elevated lactate levels in the blood of animals with irradiation-induced tumors (Ir&Tum group), are a sign of the metabolic adaption of tumor cells but also a pathway utilized by a variety of inflammatory immune cells\(^13\). Release of lactate from tumor cells is accompanied by acidification in the tumor microenvironment favoring tumor promotion, angiogenesis, metastasis, and tumor resistance\(^1\). The microenvironment of a tumor consists of a dynamic and complex network of cytokines, growth factors, and metabolic products. These contribute to significant alterations in cell growth, tissue architecture, immune cell phenotype and function\(^1\). LDH, one of the main actors of lactate metabolism, is released from cells in response to their damage, causing its baseline level to rise in the extracellular space and the bloodstream as well as other body fluids. As elevated LDH levels were found to be an unfavorable indicator for survival in cancer patients, it was suggested that LDH can be used as a marker of tumor aggressiveness\(^1\).

The preliminary results obtained in this experiment allow to hypothesize that, the ratio of tumor mass or tumor volume, detected by modern methods in clinic, to the values of blood lactate or LDH level, may be informative for characterization of the tumor process, indicating the prevalence of proliferative or secretory activity, or a balance between proliferative and secretory activities of tumor cells.

**Conclusions**

Total body irradiation in sub-lethal doses promoted the alterations of hematological and biochemical parameters of homeostasis in rats and provoked the appearance of benign tumors one year after. The ratio value of tumor mass to blood lactate or lactate dehydrogenase levels reflects the histological peculiarities of tumors, indicating in this way the prevalence of proliferative or secretory activity, or a balance between proliferative and secretory activities of tumor process.

**Data availability**

**Underlying data**

Zenodo: Peculiarities of neoplasms, appeared after total body irradiation and homeostasis parameters in rats, https://zenodo.org/record/6855331#.YtaAYIRBzV93

This project contains the following underlying data:
- biochemistry_hematology_lactate.xlsx
- All tissue images

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**Reporting guidelines**

Zenodo: ARRIVE checklist for “Peculiarities of neoplasms, appeared after total body irradiation and homeostasis parameters in rats”, https://zenodo.org/record/6855331#.YtaAYIRBzV93

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**Acknowledgements**

We thank Sergey Mikhalovsky, Matt Illsley, Levchenko, Konstantin Levchenko for assistance and useful discussion.

**References**

1. Bespalov VG, Alexandrov VA, Semenov AL, et al.: The inhibitory effect of meadowsweet (Filipendula ulmaria) on radiation-induced carcinogenesis in rats. Int J Radiat Biol. 2017; 93(4): 394–401. PubMed Abstract | Publisher Full Text

2. Hollander CF, Zurcher C, Broerse JJ: Tumorigenesis in high-dose total body irradiated rhesus monkeys—a life span study. Toxicol Pathol. 2003; 31(2): 209–213. PubMed Abstract | Publisher Full Text

3. Thomas GA, Symonds P: Radiation Exposure and Health Effects - is it Time to Reassess the Real Consequences? Clin Oncol (R Coll Radiol). 2016; 28(4): 231–236. PubMed Abstract | Publisher Full Text | Free Full Text

4. Gilbert ES: Ionising radiation and cancer risks: what have we learned from epidemiology? Int J Radiat Biol. 2009; 85(6): 467–482. PubMed Abstract | Publisher Full Text | Free Full Text

5. Baker KS, Leisening WM, Goodman PJ, et al.: Total body irradiation dose and risk of subsequent neoplasms following allogeneic hematopoietic cell transplantation. Blood. 2019; 133(26): 2790–2799. PubMed Abstract | Publisher Full Text | Free Full Text

6. Kiang JG, Olabisi AO: Radiation: A poly-traumatic hit leading to multi-organ injury. Cell Biosci. 2019; 9: 25. PubMed Abstract | Publisher Full Text | Free Full Text

7. Nakamura N: A hypothesis: radiation carcinogenesis may result from tissue injuries and subsequent recovery processes which can act as tumor
promoters and lead to an earlier onset of cancer. Br J Radiol. 2020; 93(1115): 20190842. 

Published Abstract | Publisher Full Text | Free Full Text

8. McIntyre N, Rosalki S: Biochemical investigations in the management of liver disease. In: McIntyre R, editor. Oxford Textbook of Clinical Hepatology. Oxford, England: Oxford University Press; 1991: 293–300.

9. Lotfi SA, El-Kabany H: Therapeutic Response of Black Tea Extract on Maintenance Pancreas and Intestine of Gamma-irradiated Rats. J Rad Res Appl Sci. 2012; 5(3): 619-622. 

Reference Source

10. Dainiak N: Hematologic consequences of exposure to ionizing radiation. Exp Hematol. 2002; 30(6): 513-528. 

Published Abstract | Publisher Full Text

11. Bertou J, Patel SA, Simon MC: The impact of O2 availability on human cancer. Nat Rev Cancer. 2008; 8(12): 967-975. 

Published Abstract | Publisher Full Text | Free Full Text

12. Vaupel P: Hypoxia and aggressive tumor phenotype: implications for therapy and prognosis. Oncologist. 2008; 13 Suppl 3: 21-26. 

Published Abstract | Publisher Full Text

13. Siemann DW, Hill RP: Quantitative changes in the arterial blood gases of mice following localized irradiation of the lungs. Radiat Res. 1983; 93(3): 560-6. 

Published Abstract | Publisher Full Text

14. Parks SK, Mueller-Klieser W, Pouyssegur J: Lactate and Acidity in the Cancer Microenvironment. Annu Rev Cancer Biol. 2020; 4: 141-158. 

Published Abstract | Publisher Full Text

15. Harmon C, O’Farrelly C, Robinson MW: The Immune Consequences of Lactate in the Tumor Microenvironment. Adv Exp Med Biol. 2020; 1259: 113-124. 

Published Abstract | Publisher Full Text

16. de la Cruz-López KG, Castro-Muñoz LJ, Reyes-Hernández DO, et al.: Lactate in the Regulation of Tumor Microenvironment and Therapeutic Approaches. Front Oncol. 2019; 9: 1143. 

Published Abstract | Publisher Full Text

17. Pérez-Tomás R, Pérez-Guillén I: Lactate in the Tumor Microenvironment: An Essential Molecule in Cancer Progression and Treatment. Cancers (Basel). 2020; 12(1): 3244. 

Published Abstract | Publisher Full Text | Free Full Text

18. van Wijpe S, Koornstra R, den Brak M, et al.: Lactate dehydrogenase: a marker of diminished antimutation immunity. Oncoimmunology. 2020; 9(1): 1731942. 

Published Abstract | Publisher Full Text | Free Full Text

19. Payen VL, Mina E, van Hee VR, et al.: Monocarboxylate transporters in cancer. Mol Metab. 2020; 33: 48-66. 

Published Abstract | Publisher Full Text | Free Full Text

20. Liu Y, He C, Huang X: Metformin partially reverses the carboplatin-resistance in NSCLC by inhibiting glucose metabolism. Oncotarget. 2017; 8(43): 75206-75216. 

Published Abstract | Publisher Full Text | Free Full Text

21. Keiding R, Hörder M, Gerhardt Denmark W, et al.: Recommended Methods for the Determination of Four Enzymes in Blood. Scand J Clin Lab Invest. 1974; 33(4): 291-306. 

Published Abstract | Publisher Full Text

22. Schumann G, Bonora R, Ceriotti F, et al.: IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clin Chem Lab Med. 2002; 40(7): 718-724. 

Published Abstract | Publisher Full Text

23. Foo AY, Bais R: Amylase measurement with 2-chloro-4-nitrophenyl maltooligoside as substrate. Clin Chim Acta. 1998; 272(2): 137-147. 

Published Abstract | Publisher Full Text

24. Amador E, Dorfman LE, Wacker WE: Serum lactic dehydrogenase activity: an analytical assessment of current assays. Clin Chem. 1963; 9(4): 391-399. 

Published Abstract | Publisher Full Text

25. Michaylova V, Ilkova P: Photometric determination of micro amounts of calcium with arsenazo III. Analytica Chimica Acta. 1971; 53(1): 194-198. 

Publisher Full Text

26. Jaffe M: Ueber den Niederschlag welchen Pikrinsäure in normalen Harn erzeugt und über eine neue reaktion des Kreatinins. Z Physiol Chem. 1886; 10: 291-300.

Published Abstract | Publisher Full Text

27. Czob R, Barthelmai W: Enzymatische Bestimmungen der Glucose in Blut, Liquor und Harn. Klin Wschr. 1962; 40: 585-589. 

Published Abstract | Publisher Full Text

28. Daly JA, Ertingshausen G: Direct method for determining inorganic phosphate in serum with the “CentrifiChem”. Clin Chem. 1972; 18: 263-265. 

Published Abstract | Publisher Full Text

29. Talke H, Schubert GE: Enzymatic urea determination in the blood and serum in the warburg optical test. Klin Wochenschr. 1965; 43: 174-175.

PubMed Abstract | Publisher Full Text

30. Fossati P, Principe L, Berti G: Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem. 1980; 26(2): 227-231. 

PubMed Abstract | Publisher Full Text

31. Goldschmidt M, Peña L, Rosatto R, et al.: Classification and grading of canine mammary tumors. Vet Pathol. 2011; 48(1): 117-31.

PubMed Abstract | Publisher Full Text

32. Schwartz MK: Lactic dehydrogenase. An old enzyme reborn as a cancer marker? Am J Clin Pathol. 1991; 96(4): 441-3.

PubMed Abstract | Publisher Full Text

33. Forkasiewicz A, Dorociak M, Stach K, et al.: The usefulness of lactate dehydrogenase measurements in current oncological practice. Cell Mol Biol Lett. 2020; 25: 35.

PubMed Abstract | Publisher Full Text | Free Full Text

34. Ellis ID: Intraductal proliferative lesions of the breast: morphology, associated risk and molecular biology. Mod Pathol. 2010; 23 Suppl 2: S1-S7.

PubMed Abstract | Publisher Full Text

35. Garau MMM, Calduch AL, López EC: Radiobiology of the acute radiation syndrome. Rep Pract Oncol Radiat. 2011; 16(4): 123-30.

PubMed Abstract | Publisher Full Text | Free Full Text

36. Krishnamurthy VR, Baird BC, Wei G, et al.: Associations of serum alkaline phosphatase with metabolic syndrome and mortality. Cell Mol Biol Lett. 2020; 25: 35.

PubMed Abstract | Publisher Full Text | Free Full Text

37. Wang X, Li Y: The disruption of hematopoiesis in tumor progression. Blood. 2019; 11(1): 88-91.

PubMed Abstract | Publisher Full Text | Free Full Text

38. Wang M, Zhao J, Zhang L, et al.: Role of tumor microenvironment in tumorigenesis. J Cancer. 2017; 8(5): 761-773.

PubMed Abstract | Publisher Full Text | Free Full Text

39. Snezhkova EA, Voronina OK, Zadvornyi TV, et al.: Peculiarities of neoplasms, appeared after total body irradiation and homeostasis parameters in rats. 2022. http://www.doi.org/10.5281/zenodo.6855331