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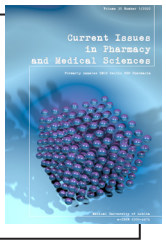

The risk of increasing tumor malignancy after PET diagnosis

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ABSTRACT

This manuscript reviews evidences underlying the estimation of risk of malignancy enhancement of advanced aggressive cancers as a result of the gamma radiation emitted by tracers used in PET diagnostics. We conclude that among many cancers, such a phenomenon likely occurs, particularly in tumor cells with an aggressive biology in the advanced stages of their development, *e.g.* prostate cancer, melanoma and colorectal cancer. Moreover, we surmise based on gathered evidence that fluorine -18 (^{18}F) labeled pharmaceuticals (^{18}F-deoxyglucose and ^{18}F-choline), commonly used in positron emission tomography (PET) can lead to malignancy enhancement of diagnosed cancer, manifesting as accelerated infiltration of the neighboring tissue, accelerated metastasis and/or radio- and chemotherapy resistance. In this review, some suggestions on future studies verifying this concept are also proposed. If our concerns are justified, it might be appropriate in the future to consider this assumption at the stage of deciding whether to undertake PET monitoring in some patients with advanced aggressive cancer.

INTRODUCTION

The initiation of cancer comes about by the long-term accumulation of adverse cell mutations before the recognized onset of the disease. Each mutant cell can give rise to the development and growth of a sub-population called a clone. Clonal diversity of neoplastic cells is related to their diverse phenotype, increased activation of glycolysis, malignant character and reduced sensitivity to anticancer therapy [1]. The clonal theory of cancer development was first formulated in the 70's by P. Nowell, and, nowadays, has been fully confirmed thanks to new techniques of cancer cell DNA sequencing. It seems very likely that in clones with a malignant phenotype, stimuli such as chemicals or ionizing radiation will cause further mutations at a lower threshold.

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Despite the effect of hypoxia on cancer malignancy acceleration being well established [2-5], the radiation caused by fluorine -18 (^{18}F) labeled tracers has not yet been fully studied. That ^{18}F labeled tracers used in positron emission tomography (PET) may be responsible for malignancy enhancement of diagnosed cancer is likely because gamma radiation triggered by ^{18}F in the cancer cell may be three orders of magnitude higher than the energy triggering water radiolysis, which leads to the free radicals formation and DNA damage [6,7]. Such high energy in cancer cell results mainly from the enormously higher uptake of ^{18}F labeled pharmaceuticals by cancer cells (named: the "Warburg effect") versus normal cells, which is the basis of PET diagnostics [8]. As a side note, recent studies have shown that the Warburg effect occurs not only in cancer cells, but also in normal cells with intense proliferation [9].

The indirect confirmation of the assumptions that the diagnostic level of radiation can enhance cancer malignancy is supported by results demonstrated by Miglioretti *et al*. [10]. They have found a statistically higher risk of mortality in women after the diagnosis of breast cancer that was caused by X-rays during screening tests of digital mammography. These studies revealed that the radiation dose used for diagnostic mammography screening in 100,000 women is enough to cause 125 cases of breast cancer (resulting in 16 deaths). It is worth noting that, statistically, screening prevents 968 deaths from breast cancer (per 100,000 women). Thus, the purpose of this review is not to undermine the legitimacy of diagnostic tests aimed at detecting the disease, but to consider whether multiple repetition of PET tests in patients with aggressive significantly advanced cancer will not enhance the severity of the mutation and malignancy of the tumor.

The results of the study of Miglioretti *et al*. [10] suggest that the level of medically accepted X-ray radiation is already able to cause cancer during diagnostic tests. Thus, it seems more likely that ionizing radiation can easier induce further mutations in the cells of an aggressive, naturally advanced tumor in which mutations arise in a spontaneous manner. In addition, it is worth emphasizing that the gamma radiation emitted in the cell in the presence of ^{18}F has similar characteristics to X-rays.

PET DIAGNOSTICS

Application in oncology

The PET technique is a common, highly sensitive imaging method used in clinical practice. PET imaging has become a standard component of oncology diagnosis and it is also applied for specific neurological and cardiovascular indications as well [11]. ^{18}Fluoro-labeled 2-deoxyglucose (^{18}FDG) is used as a PET radiotracer. Beyond ^{18}FDG, there are many other substances employed in cancer detection (among others, ^{11}C and ^{18}F labeled choline in prostate cancer detection or ^{18}F-NaF in bone metastases) [12].

Biochemical aspects

In patients with malignant solid tumors, 18FDG is the most widely used tracer in PET/CT imaging. The selective accumulation of FDG in various histological tumor cells is dependent on both Warburg and hypoxia effects [13-17]. The Warburg effect involves activation of glycolysis, even at proper, physiological oxygen levels (normoxia). In tumor cells, hypoxia takes place when the rapid dividing tumor cells phenomena is not followed by neoplasia angiogenesis, thus, the blood supply to the tumor is reduced. In this case, glycolysis activation via HIF1α enforces an increased glucose transport into the cells, mainly by membrane glucose transporter (GLUT). The consequences of these phenomena is the increase of glucose uptake by tumor cells, even more than ten times greater comparing to that of normal cells [18].

The ^{18}FDG uptake follows that of native glucose and depends on the expression of GLUT. GLUT transporters have a low transport specificity and they do not distinguish glucose from its radiolabeled analogue – ^{18}FDG. In the cytoplasm, ^{18}FDG is phosphorylated to FDG-6-phoshate, but in contrast to native glucose, ^{18}FDG-6-phoshate does not enter the glycolytic pathway and is trapped within the cell in an unchanged form [19]. The degree of selective accumulation of ^{18}FDG in tumor cells is the basis of distinguishing the PET signal from the normal tissue background, which also uptakes ^{18}FDG, but to a much lesser extent. However, the sensitivity of ^{18}FDG radiotracer in PET/CT is lower in prostate cancer. The prostate cancer cells are characterized by overexpression of choline kinase, which is responsible for the production of cell membrane components in which both acetate and choline are incorporated. Consequently, other radiotracers, for instance, ^{11}C-acetate, ^{11}C-choline and ^{18}F-choline, are used.

Several studies have found that choline PET/CT has a greater sensitivity and specificity than ^{18}FDG PET/CT in prostate cancer [20-23]. We assumed that this intensified accumulation of both ^{18}FDG or ^{18}F-choline in tumor cell may induce water radiolysis, and, subsequently, DNA damage and cell signaling that lead to increased tumor malignancy.

The basic question for the issue under consideration is whether the energy emitted by the ^{18}F radiotracer is sufficient to cause water radiolysis. Based on current knowledge, we can conclude that there is such a risk. The emitted energy of gamma rays triggered by ^{18}F in cell is much higher than the energy required to ionize the water and to consequently induce DNA damage.

The gamma-radiation energy released in the cell from ^{18}F during annihilation is over 500 keV [24], while the minimum energy at which radiolysis of water occurs is in the range of 16-20eV [7,25,26]. Therefore, the energy of radiation coming from the ^{18}FDG is about 25.000 times higher than the energy needed for free radical generation and DNA damage via water radiolysis. Furthermore, this energy may increase in the tumor cell even more than ten times more due to the above mentioned specific glucose/^{18}FDG uptake related to glycolysis activation mechanism via the Warburg or hypoxia effects [18].

The ^{18}FDG is an analog of glucose, and, similarly to glucose, is taken up by the cells, and, as glucose, is phosphorylated to the ^{18}F-FDG-6-phoshate that is a counterpart to glucose-6-phoshate. Phosphorylation does not allow both glucose and its analog to be released from the cell.

Beyond the aforementioned, there is another mechanism responsible for higher retention of ^{18}FDG. Accordingly, the 2-hydroxyl group in glucose is needed for further glycolysis, but ^{18}FDG is devoid of this 2-hydroxyl group. Thus, ^{18}FDG cannot be further metabolized in the cell up to ^{18}F radioactive decay [27,28].

The increase retention of ^{18}F not only allows for PET diagnostic, but enhances the risk of mutation, especially in aggressive cancer cells (as these are extremely susceptible to damage). Thus, it increases the risk of conversion to a tumor with even greater malignancy. The malignancy may manifest by accelerated invasion into the neighboring tissues or by metastasis and radio- and chemotherapy resistance [3, 29-32].

While the half-life of ^{18}F is only 108 minutes, the physico-chemical and chemical stages of water radiolysis last 10^{-15}, 10^{-12} and 10^{-6} s, respectively, producing e^-_{aq}, H^*, HO^*, HO_2^*,OH^-, H_3O^+, H_2, H_2O_2 that are very reactive [26].

The damage risk of even normal tissue by ^{18}F was noticed by the Medicine & Healthcare Products Regulatory Agency. The agency recommends patients to avoid any close contact with young children or pregnant woman for up to 12 hours from injection, until full decay of ^{18}F [19]. However, up to date researchers have not focused on ^{18}FDG safety in the aspect of the aggressiveness enhancement of tumor malignancy.

So far, studies have focused on the chemical, but not the physical aspects of ^{18}FDG toxicity. To resolve this dilemma we can look at the Som's *et al.* studies using nonradioactive FDG [33]. They did not noticed any evidence of acute or chronic toxicity in mice using 1000 times the human tracer dose (HTD) per wk for 3 wks, and in dogs using 50 times HTD per wk for 3 wks. Moreover, no literature was found on comparative studies of ^{18}FDG and FDG that would explain of the nature of toxicity – physical or chemical. However, the reproductive and developmental toxicity, genotoxicity and carcinogenicity of ^{18}FDG have not been tested up to now [19]. Still, a prospective 4-year study by Silberstein [34] indicated that PET radiopharmaceuticals have an extraordinary safety record with no adverse reactions. However, higher cancer invasiveness was not the subject of the study.

Current knowledge on the biological effects of ionizing radiation

The biological effects of ionizing radiation are divided into deterministic and stochastic. If the energy is absorbed in a relatively high range of value, the frequency and intensity of effects are proportional to the radiation dose and these effects are deemed 'deterministic'. One level of threshold cannot be given for everyone because of the varying radiation sensitivity of the organisms. Exposure of the entire body of mammals to a dose of 5-12 Gy leads to bone marrow, gastrointestinal, lung or brain damage, and finally death. Such extremely high dose in medicine is used only for locally irradiation during bone marrow transplantation. Among other examples of deterministic symptoms are: erythema, skin necrosis, hair loss, infertility, cataracts and fetal damage. However, deterministic effect can be observed not only as acute symptoms, but as delayed symptoms as well. Deterministic delayed symptoms can appear as a result of radiotherapy and manifest as cardio- and hepatotoxicity or pneumonia [35].

Stochastic effects are defined as effects that can occur with a certain probability. It is assumed here that the likelihood of an effect is dose-dependent, and it is not possible to set a lower dose limit below which the probability of an effect would be equal zero. Stochastic effects result from damage to the DNA even in one cell, which persists in the form of mutations or aberrations. These effects include mostly cancer and cardiovascular disease. They usually occur a few or even several dozen years after irradiation [35].

De novo tumor induction after ionizing radiation at doses used in therapy

A very useful, commonly applied parameter to characterizing ionizing radiation is linear energy transfer (LET). This is the value of energy left by the ionizing particle in the medium per unit-traveled length of path, and is different for different particles and different media. For large particles like alpha, in water, the value of LET is greater than 100 $keV \cdot \mu m^{-1}$, for carbon ions, the corresponding value is 10-100 $keV \cdot \mu m^{-1}$, for radiation therapy protons, the LET is around 10 $keV \cdot \mu m^{-1}$ and for electromagnetic radiation (gamma, X), the LET value is approximately up to 1 $keV \cdot \mu m^{-1}$. Thus, the energy of these individual particles exceeds 1 μm of length path in water [36,37]. However, maximum cell killing occurs at an LET of approximately 100 $keV \cdot \mu m^{-1}$, not 1000 keV/μm, and relative biological effectiveness (the coefficient applied to determine the biological effectiveness of a given radiation relative to X-rays) shows the greatest changes for LET values between roughly 20 and 100 keV/μm [38].

LET value importance can also be read in a different way – by asking how long a road is needed in a given medium (e.g. water) for particular types of radiation to leave the same unit of energy. A high LET value (high-LET) indicates low permeability of radiation particles through the medium. This means that they give out a lot of energy in a short path. Such particles, for instance, alpha (^{223}Ra), rather do not pose a threat for the body to accidental radiation from outside. However, inside the body, they can destroy all molecules in a short distance because the higher the deposited energy, the more severe the damage in cell structures localized near the particle path, including damage of DNA.

In contrast, gamma or X radiation, both of which have electromagnetic nature, have low value of LET (low-LET). Because of their nature/size they have very high permeability and do not leave much energy in the medium. For instance, during decay, the gamma radiation of ^{60}Co (commonly used in cancer radiotherapy) releases two gamma quanta of 1.17 and 1.33 MeV, but the LET value ranges between, 0.2-0.3 keV μm^{-1}; whereas ^{210}Po, an emitter of alpha particles with energy 5.407 MeV, has LET value of 140 $keV \cdot \mu m^{-1}$ [39]. Thus, LET helps to explain why radiation damage is sometimes disproportionate to the absorbed dose (unit: Gy).

High-LET leads to DNA double-strand breaks that are mostly difficult to repair, and result in cell death. In mice studies, following exposure to high-LET radiation, immortalized human cells undergo malignant transformation until they become tumorigenic [40]. Significant changes in signaling pathways have also been revealed [32]. In contrast, cell death caused by low-LET, is rare because repair mechanisms cope with the amount of damage that had occurred [41]. However, cell exposure to an even lower energy of ionizing radiation leads to the formation of free radicals (water radiolysis) resulting in damaged DNA, as well as damage in the cytoplasmic organelles, including the endoplasmic reticulum. Furthermore, the effects of radiation-dependent changes in the signalling pathways are not just intracellular, but occur between cells via gap junction [42,43]. Hence, neighbouring cells undergo DNA damage in a similar way as directly irradiated cells, and single and double-strand breaks, sister chromatid exchange and diminish of DNA methylation may lead to genomic instability [40,44].

Local irradiation during cancer therapy ranges from 2 to 80 Gy in divided repeated doses forms of 1.5 to 2.5 Gy, and might be associated with secondary cancer development [45]. Secondary cancer may appear as totally new tumors in

other organs than that which were documented in the past 20 years. For instance, it is well established that as a consequences of radiotherapy in some percent of patients suffering from prostate cancer, bladder cancer (which is characterized by unfavorable prognosis) may develop [46,47]. There is also a significant increase in cases of myeloid leukemia within a few years after radiation therapy of breast cancer patients. A further complication of radiation therapy for breast cancer patients is potential esophageal cancer and lung cancer (observed in about 2% of all cases) [48,49].

The above examples reveal that the medical application of radiation can lead to the *de novo* development of new histological tumors in other organs. Moreover, it clearly indicates the complications of radiotherapy, in which relatively high radiation energy levels are used locally. However, our intention in this article is to discuss the question as to whether is it possible that the much lower level of radiation energy that is used in PET diagnostics could lead to an increase in the aggressiveness of diagnosed cancers. Thus, first of all, it must be stated that rarely do radiation therapeutic doses initiate *de novo* cancer development (in normal cells). Still, the question arises as to whether at diagnostic PET levels of radiation (which are much lower than the therapeutic), the biology of existing tumors might worsen. To resolve this issue, we must know the answers to the following physical and biological questions:

1. Is the gamma radiation energy released in ^{18}FPET diagnostics sufficient to cause genetic changes?
2. Is it easier to develop mutation after radiation in normal cells or in cancer cells of the same tissue?
3. Will the same probability of mutations have the same consequences in normal cells, early stage cancer cells (monoclonal tumors) and advanced phase cancer cells – tumors with a very large number of different clones?

Possibilities of adverse effects at diagnostic level of radiation

In clinical conditions, in the normal lymphocytes of patients who have been diagnosed by CT (range of dose 0.01-2 Gy), mutations have been found [50,51]. Moreover in Kempf's study, a relatively low dose of radiation, similarly to that used at diagnostics CT levels reveal changes even in normal cells [52]. In addition, cell proteome and transcriptome changes and neurological disorders in hippocampus and cortex after 24 h low-LET – gamma irradiation (0.1 or 0.5 Gy) has been observed. The investigation also showed that synaptic functions and signaling pathways directed by mitochondria were changed, which was clearly seen in the cortex at 0.1 Gy. If such changes are observed in normal cells, it is much more probable that the same energy disturbs the new equilibrium acquired in cancer cells.

The commonly used PET positron emitters are characterized by maximum emitted energy and maximum distance range in water that are for ^{18}F, respectively, 0,34 MeV and 2,4 mm [53]. Energy released from ^{18}F is not only the result of the positrons emission (β+, Emax = 634 keV), but subsequent annihilation photons that produce γ-rays with energy of 511 keV. During a typical clinical protocol involving the administrationof 350-750 MBq ^{18}FDG, most tissues are irradiated throughout the patient's body at an averae of 4-9 mGy [24]. However, in some normal tissues with higher metabolism, the concentration of intracellular ^{18}FDG are much higher, thus gamma radiation can be consequently much higer (13-233 mGy) as well [54-59]. The above-mentioned range of radiation doses are observed in normal tissue and as you can see in some cases, the level of radiation reaches doses as are observed in a CT scan. However, in neoplasia cells, the doses are certainly higher because of the specific accumulation related to Warburg and tumor hypoxia effects [60].

The absorbed radiation level in cancer cells depends on the glucose requirements of the tissue. This can be even higher than one order of magnitude greater than the normal tissue cell from which the tumor originates. [18]. The development of the tumor process at the cellular level is accompanied by an increase in the expression and activity of the glucose transporter (GLUT1). As GLUT1 is not chemically specific, the amount of ^{18}FDG transported into the cells is usually much higher in cancer cells. Moreover, when ^{18}FDG is phosphorylated by hexokinase, its metabolite ^{18}FDG-6-phosphate cannot pass by the glycolytic pathway as it is not a substrate for the next enzyme. As a result, ^{18}FDG-6-phosphate is essentially trapped within the tumor cell and may be imaged divergently compared to the normal cells by PET [61,62]. According to Mettler *et al.* [63], ^{18}FDG PET scans transmit one of the highest effective doses to patients (14.1 mSv). To compare radiation dose, for instance, radiation for chest X-ray reaches merely 0.02 mSv nd 6.5-8 mSv for a CT scan of the chest [*64]*. On average, during one PET test, the patient receives the dose he would receive during 1200 chest X-rays.

Certain research groups have identified that fractional dose rates generate more damage than fixed low dose rates [65] and the damages resulting from a very low dose rate (94 mGy/h) evade DNA damage surveillance mechanisms [66]. Indirect evidence suggests that ^{18}FDG accumulation in cancer cells may confer tumor aggressiveness. According to Maddalena *et al.* [27], Mankoff *et al.* [67] and Folpe *et al.* [68], there is a relationship between the level of FDG accumulation and histological grade in lung, brain, hepatic and musculoskeletal cancers. Thus, it is rational to state that the higher the ^{18}F accumulation, the greater the risk of malignancy elevation.

There are few contradictory studies about the biological effect of low dose X rays. They concern the biological effectiveness of low energy X-rays used for mammography breast screening. Still, recent radiobiology studies have provided compelling evidence that low energy X-rays may be 4.42 ± 2.02 times more effective in causing mutational damage than higher energy X-rays [69]. Miglioretti *et al.* [10], for example, have generated estimations for the potential harm from radiation exposure of screening strategies for breast cancer. Therein, Miglioretti *et al*. calculated the energy of radiation coming from the ^{18}FDG to be about 25.000 times greater than the energy needed for free radical generation via water radiolysis and DNA damage. What is more, mammography was determined to be affected by dose variability, initiation age and screening frequency. Indeed, their study suggests that women with large breasts receive greater radiation doses and may have a greater risk for radiation-induced breast cancer affecting cancer cells. Taking it all together,

it seems that irradiation energy released from ^{18}FDG during PET diagnosis is sufficient to induce changes, especially in cancer cells where it is concentrated, and so can bring about DNA mutation much easier than in the normal cells from which the tumor originates.

Factors that might strengthen malignancy of cancer

In attempting to answer the previously asked questions whether it is easier to generate a mutation after radiation in normal cells or in cancer cells of the same tissue – from physical point of view, the probability of cell changes at the same dosages is similar in normal and cancer cells. However, ^{18}FDG concentration in most cancers is one or more orders of magnitude higher than in normal cells. Thus, for that reason alone there is much higher probability of mutation in cancer cells than in normal cells.

Finally, the answer to the key question: will the same probability of mutations or other signaling changes have the same consequences in normal cells, early stage cancer cells (monoclonal tumors) and advanced phase cancer cells – tumors with a very large number of different clones? To date, hypoxia is a well-recognized factor exemplifying the diverse response of normal and cancer cells – a well-known factor intensifying the malignancy of tumor. Oxygen deficiency arises in the area of the tumor as a result of the intensive proliferation of cells whereby the cells of the blood vessels are not able to keep up their division. The result of adaptive changes under hypoxia is the increasing clonal heterogeneity of the tumor cell. The clinical significance of these changes is well established [70]. This is the increasing dynamics of invasion into the surrounding tissues, systemic spreading [71-73] and varied clonal sensitivity (or its insensitivity) to drugs [30,74,75].

In a simplification, we can say that a differentiated response of individual clones to drugs is essential for treating them as separate disease entities within the same histological tumor. Thus, adaptative response to hypoxia is divergent for normal and cancer cells. A surviving cancer cell is that which adapts better to the adverse environment. Moreover, cancer cell adaptation makes them very much aggressive and better expansion of cell neoplasia is seen as compared with the normal cells of the surrounding normal tissue. In addition, normal cell under hypoxia do not intensify their proliferation as do cancerous ones.

In this study, we surmised that a similar divergent effect in cancer and normal cells would appear if the same probability of DNA mutation exists in both type of cells. Let us also conjecture that a given dose of gamma radiation is enough not only to induce mutation or other signaling changes in not only cancer cells, but in normal cells as well. Thus, the consequences in normal cells, early stage cancer cells and advanced phase cancer cells will be different. This is an assumption because, according to current knowledge, there is no published data about the impact of the ^{18}F tracer on tumor aggressiveness, while attempts were made to follow such an effect in normal cells with high index of proliferation [54]. Our justification can only be based on theoretical considerations. However, it seems very likely that consequences of repair DNA genes mutation allows cancer cells to gain higher autonomy, proliferation and consequently higher cancer aggressiveness in comparison to normal cells.

Future studies

To verify the main thesis of this paper it would be rational to conduct the study at *in vitro* level. It seems that research using cell lines of highest aggressive cancer will be the best approach. What is more, different kinds of cell lines with extreme different stages of the disease degree and cytological differentiation (primary tumor, metastasis) should be compared in terms of their ability to acquire an aggressive metabolic phenotype after incubation with ^{18}F-labeled pharmaceuticals. It should be noted that the control and repairing mechanism in such cells is out of order and subsequently enhancement of gamma ray-induced malignancy is more probable than in early stage cell precursors of cancer or normal cells. However, even in the normal cells of the hippocampus and cortex, proteome and signaling pathways related to mitochondrial and synaptic functions are changed at doses much lower than cancer cells during exposure to ^{18}F. Beyond the aforementioned, the effects of the biological impact of ^{18}F should be tested after different number of passages. During such studies, a pool of cells should be separated and re-treated with ^{18}F to reflect patient re-examination with ^{18}FPET in order to verify the effectiveness of treatment. After the subsequent cell passages, the progress in malignancy at the molecular, functional and morphological levels should be assessed. Tests that reflect tumor aggressiveness/malignancy in humans, e.g.: proliferation assessment, migration and invasion analysis of gene expression profile associated with an aggressive phenotype of tumor cells and chemo- and radio sensitivity can be used.

CONCLUSION

The key assumption of our debate is that ^{18}F-labeled tracer can enhance high malignancy in advanced stage cancers during PET diagnostic testing. Our thesis is that the higher mutagenic effects of ionizing radiation, the greater clonal diversity of the tumor and, consequently, aggressiveness of cancer cells. According to this thesis, cancer cells at the highest stage of tumor development will be much more vulnerable to gamma ray – induced aggressiveness changes than their precursors at earlier stages of tumor development. In the work, we pointed out that the energy of radiation coming from the ^{18}FDG is about 25.000 times greater than the energy needed for free radical generation via water radiolysis and DNA damage. The main concept is supported by the fact that ^{18}FDG concentrations for most types of cancers are one or more order of magnitude higher than in normal cells – this is foundation of PET test concept. However, it seems very likely that consequences of mutation of DNA repair genes allow cancer cells to gain higher autonomy, proliferation and consequently higher cancer aggressiveness than normal cells. Thus, according to our suppositions, even at diagnostic doses of ^{18}FGD, the radiation can stimulate existing tumors to gain more aggressive features, manifesting as intensification of neighbouring tissue infiltration and worsening of metastasis. We, therefore, proposed a direction of future studies to firmly confirm our conclusions.

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DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included within the article.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests.

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