Author's response to reviews

Title: DNA damage response and DNA repair - dog as a model?

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Author's response to reviews: see over
Dear Mr Nazareno, dear Journal Editorial team

We were not very happy to see that you missed to pass on reviewer’s comments! It is somewhat more work to do this sequentially. Anyhow, we would like to thank you and the reviewers for the valuable comments concerning the structure and content of the reviewed publication. Enclosed you will find the revised paper. We carefully considered the reviewers comments and responded to them as lined out in the following paragraphs.

The individual comments of the reviewer have been addressed as follows:

**Reviewer 1:**

The language should be improved and careful spelling and check should be performed

A native-language English colleague again revised the language and careful spelling checks were performed.

1. The study on DNA damage and DDR should involve and especially in the case of radiation therapy comparison the idea of clustered DNA damage. This the authors can combine with recent findings for BRCA1, DNA-PK involvement not only in DSB repair but also other repair pathways like BER and processing of non-DSB lesions (Peddi et al. Free Radic Biol Med, 48: 1435-1443, 2010 and references therein).

   This is a valid point from Reviewer 1, however the aim of this Debate-Article is to compare repair systems and DDR pathways between humans and dogs in general. We did not solely mention the treatment form of radiation in this article and keep it more general as most of the studies with dog cells use other agents than radiation (e.g. etoposide, cisplatin, UV). There are no studies available on canine cells and clustered DNA damage therefore we think this cannot be included and would also reach out of the aim of this article.

2. There is a lack of clear data by the authors on the suitability of using canine models with respect to radiation effects in the case of tumor treatment. The authors shall include a discussion if available data.

   To the best of our knowledge there are no studies on canine models and DNA damage after radiation. Furthermore we do not specifically discuss the option of radiation treatment here. We agree, there is a general lack of knowledge and while we aim to contribute to establishing the data and the knowledge in the future, currently we cannot present data.
3. A Table with the similarities and differences between canine and human in DDR (genes, expression of proteins, oncogenes etc..) and cancer incidence should be made. There are not so many studies involvin canine models and should be important.

We have decided to show important players below our Figure 1: „important players of DNA repair mechanisms mutated or misregulated in both canine and human cancers are depicted in the lower part“ (see legend). While we could put together a table including also the oncogenes, however we already had to shorten our paper length and reference list in a prior version of this manuscript. Including a new list with oncogenes would again elongate the publication.

4. Is there any specific study like in canine carcinogenesis steps as in the case of human colon cancer and which genes maybe involved?

We have mentioned a possible equal tumorigenesis between human and canine CML with the BCR-ABL gene translocation as the driver (marked in red on page 6). In the new part of chromosomal instability we also mention canine colorectal cancer where the amount of chromosome alterations correlates with tumor stage as in human colorectal cancer (in red, page 4, chapter 2).

5. The authors shall discuss the possible problems in using canine models for example ethical issues, expensive setup etc.? Is there finally a necessity which can be rationalized?

We postulate the use of dogs with spontaneous tumors, presented for treatment of their disease. „Over 1 million of pet dogs are diagnosed annually and managed with cancer in the United States [16], and these patients can often be entered in clinical trials when conventional treatments do not meet the goals of the veterinary oncologist“.

6. Chromosomal instability is accepted as a key step in cellular transformation and cancer. What do we know in canine models?

At the end of chapter 2 we mention genomic instability (shown in red): „Consequently, the extent of genomic instability has been described to be equally comparable in certain canine and human tumor types, such as osteosarcoma and colorectal cancer [39, 40]“. As we had to shorten our article in the beginning, we cut out the following explanation: „For colorectal cancers (CRC) it was shown that the amount of chromosome alterations correlates with tumor stage in both species to the same extent [57]. In human CRC only 13% show microsatellite instability (MSI), which is due to mismatch repair defects [58]. Further, the majority of human tumors display chromosomal instability (CIN), as do the canine CRCs [57,59]. This indicates an equal distribution of hereditary and sporadic CRC within both species. Human osteosarcomas are also highly genetically unstable and therefore contain many chromosome aberrations [60]. Maeda et al. showed for ten canine primary osteosarcoma samples that the chromosome count is always aberrant [56]. This phenotype was also verified in several canine osteosarcoma cell lines and tumor samples [56,61]. To shed more light on tumor-associated defects, further investigation of canine tumors with regard to their mutation status and CIN would be needed.“

We could include this part again but we think that this is too long and not the scope of the article and the reader could read the references 39 and 40 when interested.
Review 2:

Reviewer 2: (minor essential revisions)

1- Background: When the authors state “mouse xenograft tumors revealed a second wave of dsbs,... 2 days after the initial wave”, they should specify that they are referring to radiation-induced DNA break, if it is the case.

Clarification is now provided adhering to the referenced publication: „The cause of this second, unexpected wave of dsbs is still unknown, with suspected causes of radiation induced genetics instability and apoptosis“.

2- Background: Please define in the text the acronym “IR”. I believe that it was not previously done.

Definition is now provided

3- Background: The authors state “As the overall lifespan of dogs is shorter than that of humans, conclusions from clinical studies can be drawn faster”. It is true that the evolution of most cancers in dogs is often much faster than in humans, but that is a fact per se, not necessarily because their life span is shorter.

This statement has been adapted to the reviewer’s suggestion

4- Discussion- question (1): The statement “Nonetheless, the canine gene products seem to be more closely related to their human homologs than those of mice.” would benefit if a couple of examples are provided.

A suitable example was included: „As an example, the BCR-ABL fusion gene could be detected with fluorescence in situ hybridization (FISH) in canine chronic myelogenous leukemia (CML) and chronic monocytic leukemia, which is equivalent to the Philadelphia chromosome (with the BCR-ABL fusion) in human CML showing equal genomic break sites (Ref(s))”

5- Discussion- question (2): The authors state “throughout mammalian species, deregulated cell cycle check points and apoptosis mechanisms lead to: increased proliferation (...) by the continuously activated DDR.” It is debatable if the activated DDR increase proliferation per se. Considering that its activation succeeds a cycle arrest in order to determine cell viability, it could be hypothesized that it would reduce, not increase, proliferation. Of course that if DDR is deregulated, then the effect would be increased proliferation, but its continuous activation by itself does not guarantee deregulation.

The statement has been reworded appropriately to the reviewer’s suggestion.

6- Discussion- question (3): Replace “Study addressing the capacity...” by “One
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study addressing the capacity...”

Replacement has been performed

7- Discussion- question (3): The sentence “In normal vs. adenoma samples, BRCA2/1 and RAD51 expression was reduced” is misleading since it means that normal tissues have less expression than adenomas. In the reference, however, the opposite result is described: “The relative copy numbers of BRCA1 in adenomas of 6 of 10 dogs was reduced to <0.5-fold when compared with normal gland epithelium (Fig. 5). Similarly, expression levels of BRCA2 and RAD51 were also reduced in adenomas of 4 of 10 dogs” (sic ref 45)

The proposed (correct!) wording is now provided: „In adenoma vs. normal samples, BRCA2/1 and RAD51 expression was reduced.“

8- Discussion- question (3): Please provide units in “Though dogs live shorter than humans (16.6 vs. 90, respectively)...”

Units are now provided

9- Discussion- question (4), skin cancer: The authors state “In the two most common tumors of the skin, squamous cell carcinoma and melanoma...”. However this is not true. Many of the most frequent canine skin tumors are benign (lipomas, histiocytomas, perianal gland adenomas, etc.) and, amongst the malignant tumors, the most prevalent are mast cell tumors (Withrow & MacEwen’s Small Animal Clinical Oncology, 5th Ed., pp. 306).

The statement has been corrected

10- Discussion- question (4), skin cancer: In the sentences “The p53 protein was shown to solely localize to the cytoplasm in many tumor cases” and “Therefore, misregulation of important tumor suppressor genes leads to genomic instability and progression of canine skin tumors” the authors should specify that they are referring to skin melanomas, since it hasn’t been demonstrated in other skin tumor types.

The suggested addition has been made

11- Discussion- question (4), skin cancer: The statement “Important cell cycle regulators are mutated in about 72% of the tumors” is, in this reviewer opinion, oversimplified. Without tumor specifications or references, the reader may be confused about which type(s) of tumor(s) are these results.

Upon discussion amongst all authors, the provenance of this (unreferenced) statement could not be found. As it is indeed a very simplified statement, we decided to omit it in the revised version.

12- Discussion- question (4), skin cancer: In the sentence “...the high frequency of malignant lymphoma (7-24%) in dogs...” Please specify the percentage, i.e. 7-24% of all canine tumors, if it is the case (I presume it is).
The suggested specification has been made

13- Conclusions: Please write “in vivo” in italic.

corrected

14- Conclusions: Please replace “Integrating canine tumor models” by “Integrating spontaneous canine tumor models”.

The suggested replacement was performed

Having addressed all comments and integrated the suggestions of the reviewers we hope that the manuscript is now suitable for re-evaluation and/or publication.

Thank you very much in advance, yours sincerely

Carla Rohrer Bley and co-authors