Reviewer's report

Title: Inhibition of Radiation Induced Migration of Human Head and Neck Squamous Cell Carcinoma by Blocking EGF Receptor Pathways

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Reviewer: Maria Sundvall

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In the manuscript entitled “Inhibition of Radiation Induced Migration of Human Head and Neck Squamous Cell Carcinoma Cells by Blocking of EGF Receptor Pathways” Dr. Pickhard et al. study the effect of irradiation on migration and proliferation of HNSCC cell lines in vitro. They show consistent with previous studies that irradiation inhibits HNSCC proliferation. However, according to their data irradiation stimulates migration of HNSCC cells. Based on experiments with chemical inhibitors the authors suggest that tyrosine kinase activity of EGFR and its downstream pathway components, PI3K, mTor and MEK1 are involved in HNSCC migration. Since radiation therapy is widely used to treat HNSCC, data presented by Pickhard et al. is interesting and has potential clinical relevance. In general, methods they chose are valid to test their hypothesis. However, several points should be addressed before publication in BMC Cancer:

Major Compulsory Revisions

1. The authors discuss and make conclusions based on western blot data not shown. Please include these data as a new figure in the manuscript.

2. The data presented in table 1 is not clear. What is p-value 0.000? Data of another time point can be omitted for simplicity.

3. Table 2 is not very informative. Instead of presenting absorbance values of one experiment, data should be processed further and presented e.g. as bars (like migration experiments in fig 2-4). The average value of at least triplicate wells of a representative experiment with SDs should be shown. Is fig 5 already made of these absorbance values of CAL cell line in table 2? Combine fig 5 A and B to allow comparison between the data of irradiated and non-irradiated cells with or without inhibitor treatment. If all cell lines behave relatively similarly in MTT assay with or without inhibitors, I think it is enough to show only data of the one representative cell line as fig 5 and delete table 2. Since inhibitors were added 12 h prior to radiation treatment, include also time point 0 (measurement just before radiation).

4. The authors say that activation of EGFR is important for the migration of HNSCC cells after irradiation. However, they did not see any activation of EGFR by irradiation. Moreover, they show that stimulation of cells with EGFR ligand EGF inhibits rather than stimulates migration induced by irradiation. This seems inconsistent. The authors should give a putative explanation for this or provide...
additional data.

5. It is not clear how the migrating cells were counted. Representative photographic images of the wound healing assay +/- irradiation should be shown (e.g. with one cell line at one time point).

6. Given the importance and novelty of the finding that irradiation stimulates migration of HNSCC cells the basic finding should be confirmed with another type of assay, e.g. with boyden chamber migration assay, or alternatively the authors should analyze whether irradiation also promotes invasion of these cells in vitro e.g. through matrigel.

7. There is inconsistency between methods, figures and figure texts. For example, in methods section, 1st chapter, the authors state that all measurements were made in nine experiments and after culturing cells for 24 h after irradiation? To what assay is this referring? According to methods, migration assay was analyzed at 12 and 24 h time points after IR, and MTT assay measurements were made in eight experiments and incubated 12, 24 or 72h after IR? And table 2 and figure 5 show 24, 48 and 72 h time points for MTT assay? These need to be clarified.

8. The results and discussion section about western blot data should be rewritten to make it more coherent. Please check also these in Results section: i) What does the word “probe” mean in the title? Cells?; ii) Why did PD98059 associate with a decrease of phospho-MAPK at 0 time point? When was the inhibitor added?; iii) The last sentence “In all probes …” in unclear.

9. Figure 1 should be improved. For example, MAPK=Erk and PD98059 inhibits MEK1 (=MAP kinase kinase). This error needs to be corrected. The classical components of MAPK cascade should all be illustrated (Ras-Raf-MEK-Erk). Indicate also cell surface to the picture. What are the black circles attached to EGFR, Raf, MAPK and PKB?

10. More up-to-date discussion about the role of EGFR inhibitors in the treatment of HNSCC is needed in Background and Discussion sections. For example, the anti-EGFR antibody cetuximab has already been approved by the FDA and EMEA for the treatment of locally advanced HNSCC with concomitant radiotherapy. Include data and reference of the landmark phase III trial by Bonner et al. demonstrating survival benefit of concomitant use of cetuximab with radiation compared to radiation alone in the treatment of patients with advanced HNSCC in the manuscript. Discuss the current clinical status of EGFR targeting antibodies and tyrosine kinase inhibitors as well as inhibitors of EGFR downstream signaling pathway components in combination with radiotherapy for the treatment of HNSCC.

11. Please update the references. For example, reference 17 (published in 2001) is rather old to cover recent molecular strategies.

Minor Essential Revisions
1. The same terms should be used throughout the text. For example, i) when both PKB/Akt and PI3K/Akt are used it is not clear that PKB is an alternative name for Akt whereas PI3K and Akt are different components of a signaling pathway; ii) the use of alternative names for the same molecule (e.g. phospho-Erk vs. phospho-MAPK) is misleading.

2. Abstract – Results: It is misleading not to mention the results of EGF or inhibitors on irradiation induced migration.

3. Abstract – Conclusion: Specify “these” proteins in the last sentence.

4. In Background section, the 1st paragraph: “Curative and adjuvant…” sentence is unclear and should be rewritten. Describe the anti-neoplastic properties of radiation as a separate sentence.

5. In Background section, the 3rd paragraph: “Also, Camphausen et al….” sentence is unclear and should be rewritten.

6. In Background section, the 5th paragraph: In addition to references 15 and 16, other references are needed to cover the studies that correlate molecular markers with radiation response. Please add also what is known of EGFR and its downstream signaling molecules as predictive markers for radiation response in HNSCC.

7. In Background section, the 6th paragraph: The sentence “Mutations in the cellular…” is unclear and should be rewritten.

8. In figure 5 “kontrolle” -> control.


10. In Discussion section, the 3rd paragraph: “blockade of Akt by rapamycin” is not correct.

11. In Discussion section, the 5th paragraph: “stadium” -> stage

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare no competing interests.