CD44 – A Cancer Stem Cell Related Biomarker with Predictive Potential for Radiotherapy

Michael Baumann* and Mechthild Krause

Dept. of Radiation Oncology and OncoRay Center for Radiation Research in Oncology, Medical Faculty and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany

*Corresponding Author:
Prof. Dr. med. Michael Baumann
Klinik für Strahlentherapie und Radioonkologie
Universitätsklinikum und Medizinische Fakultät
Carl Gustav Carus
Technische Universität Dresden
Fetscherstr. 74
01307 Dresden
Germany
Michael.Baumann@uniklinikum-dresden.de

Summary

CD44, a cancer stem cell (CSC) related surface marker, correlates with local control after radiotherapy of early laryngeal cancer. For the first time, a CSC-related marker has been functionally validated for radiotherapy in patients. CD44 expression bears potential to predict outcome of radiotherapy by assessment of CSC density.
In this issue of Clinical Cancer Research, de Jong et al. (1) report that CD44 expression correlates with local tumour control of early laryngeal cancer treated by radiotherapy alone.

It is well recognized that the response of head and neck squamous cell carcinomas (HNSCC) to radiotherapy differs considerably between patients. Several radiobiological parameters have been identified as underlying mechanisms, including differences in the number of cancer stem cells, their cellular radiosensitivity, repopulation capacity during the course of radiotherapy and tumour hypoxia. To further improve treatment outcome after radiotherapy, predictive tests are required that allow to tailor radiation doses, fractionation regimens, the use of combined treatments or surgery to the individual patient. Several clinical predictors like tumour stage, histology and grading, have been used for a long time and are today’s basis of treatment prescription. Despite considerable progress in molecular diagnostics, so far no specific tumour biology-based predictive test has made its way into routine clinical practice.

CD44 positive HNSCC cells have been shown previously to initiate tumour growth in immunocompromised animals much more efficiently than CD44 negative cells, indicating that cancer stem cells (CSC) are enriched in the CD44 positive subpopulation of HNSCC (2). For radiotherapy, the definition of a cancer stem cell as a tumour cell that has the potential to generate a full tumour, implies that all cancer stem cells need to be killed to reach a permanent local tumour control (3, 4). Determination of permanent local tumour control as an endpoint of experimental or clinical studies currently is the only available functional assays of survival of CSC after irradiation (3, 4). Assuming a linear increase of the absolute number of CSC with increasing tumour volume, the known negative correlation of the tumour control probability with the logarithm of the tumour volume indicates that the number of cancer stem cells that need to be inactivated by irradiation is an important determinant of local tumour control (5-7)(figure 1). This is supported by seminal experiments showing a correlation between transplantability of different tumour models and the tumour control dose 50% after single dose irradiation (8). The fact that this correlation still exists when fractionated radiotherapy is used in the clinical setting, where several radiobiological parameters and intertumoral heterogeneity affect the results, implies that the number of CSC is among the dominant predictors of tumour cure after radiotherapy. This is supported by a close correlation of TCD50 after fractionated irradiation over 6 weeks with TCD50 after single dose under clamp hypoxia where only CSC number and radiosensitivity are expected to affect the result (9). Furthermore, the combination of stem cell density determined by transplantation...
assays and their intrinsic radiosensitivity has been shown to significantly predict tumour radiocurability (10).

While the importance of the number of cancer stem cells for local tumour control is obvious, until recently no marker was available that could measure the stem cell density of different tumours in the clinics. Such markers would have important potential as predictors for local tumour control. De Jong et al for the first time show in a group of patients with comparable tumour stages and treatments, that CD44 mRNA expression as well as CD44 immunohistochemical score was the only significant predictor for local tumour control when different gene signatures were compared and correction for multiple testing was used. These data of a hypothesis-driven approach were confirmed by the results of a data-driven approach, where all probes were included with at least 20% of the samples having a minimum fold change greater than 1.35 and a p-value for log-ratio variation of <0.01. Also here, the most significant marker discriminating between cures and recurrences was CD44. In addition, laryngeal cancer cell lines were evaluated. Here, the CD44 expression correlated with in vitro plating efficiency, a marker for the percentage of clonogenic tumour cells, but not with intrinsic radiosensitivity of the clonogenic tumour cells in vitro. Although clonogenic cells in vitro do not necessarily reflect cancer stem cells in vivo (4), these data support that CD44 correlates with the number and not with the intrinsic radiosensitivity of CSC.

The data published by de Jong et al. are of great relevance for translational research in radiotherapy. For the first time a clinical dataset on a CSC-related biomarker has become available that is consistent with the preclinical experiments showing the importance of intertumoral heterogeneity of CSC density for local tumour control after radiotherapy. Based on these results CD44 should be further evaluated in patients with early laryngeal cancer for its potential as predictive biomarker for individualized treatments, e.g. radiation dose escalation or primary use of surgery in those tumours judged as radioresistant. Such studies may also be used to address whether CD44 is a surrogate marker or measures CSCs directly. In the latter case, evaluation of CD44 together with tumour volumetry might allow to estimate the absolute CSC number of tumours. Radiobiologically a direct correlation is expected between the logarithm of CSC number and the dose necessary for tumour control. If the CSC number were available for a given tumor, this would in principle allow to integrate this parameter directly into dose prescription and radiation treatment planning.

Another important avenue for further research is to validate the findings made in early laryngeal cancer for other HNSCC. In contrast to early laryngeal cancer, most other HNSCC treated by radiotherapy are much larger and potentially more heterogeneous in other radiobiological parameters which determine outcome. One therefore might speculate that
CD44 may lose some of its dominance as a predictor and may assume clinically relevant potential as biomarker only when combined with other parameters, such as a quantitative assessment of hypoxia, cellular radiosensitivity, or proliferative activity.

The last decade has seen major achievements in identification of makers which may be used for accumulation of CSC, thereby increasing understanding of CSC biology. The study by de Jong and colleagues intelligently combines such technologies with analysis of local tumour control, the only clinical endpoint which is specific for CSC survival after radiotherapy. This, for the first time, functionally validates a CSC-related marker in patients, and widely opens the door for further translational research into CSC-linked biomarkers for radiotherapy.
Figure legend:

**Figure 1:** In the same individual tumour, the CSC number is expected to increase linearly with tumour volume. A higher number of CSC requires a higher irradiation dose to permanently cure the tumour. Each irradiation fraction kills a certain percentage of the CSC, while between the fractions the surviving CSCs can repopulate and increase their number.

The figure shows three different cases. In case 1, the first tumour (light blue) is small and has a given number of CSC. Alternatively, another tumour (yellow) may have a larger size but lower CSC density and thus the same low absolute number of CSC. Both tumours will be cured already after a relatively low irradiation dose (right panel). In case 2, the first tumour (light blue) is irradiated at a 100fold larger volume. The CSC density is still the same, while the absolute number is increased. The irradiation dose needed to cure this tumour is higher because of the higher number of CSC. The same dose would be needed for another tumour (orange) of a smaller size but the same absolute number of CSC. In case 3, the volume and the number of CSC in the first tumour (light blue) has again increased 100fold before start of radiotherapy. Here, the applied irradiation dose is not sufficient for permanent local tumour control, because one CSC survived. The same would apply for a smaller tumour (dark blue) with higher CSC density.
References

Case

1st tumor

Different tumor, same chance of cure

Radiotherapy fractions

Follow up

Number of cancer stem cells

Irradiation dose

Time

Macroscopic tumor

Microscopic tumor

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