

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

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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 the potential to predict the outcome of radiotherapy by assessment of CSC density. *Clin Cancer Res*; 16(21): 5091–3. ©2010 AACR.

In this issue of *Clinical Cancer Research*, de Jong and colleagues (1) report that CD44 expression correlates with local tumor 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 (CSC), their cellular radiosensitivity, repopulation capacity during the course of radiotherapy, and tumor hypoxia. To further improve treatment outcome after radiotherapy, predictive tests are required that allow tailoring radiation doses, fractionation regimens, the use of combined treatments, or surgery, to the individual patient. Several clinical predictors, like tumor stage, histology, and grading, have long been used and are the basis of current treatment prescription. Despite considerable progress in molecular diagnostics, so far no specific tumor biology-based predictive test has made its way into routine clinical practice.

CD44-positive HNSCC cells have been previously shown to initiate tumor growth in immunocompromised animals much more efficiently than CD44-negative cells, indicating that CSCs are enriched in the CD44-positive subpopulation of HNSCC (2). For radiotherapy, the definition of a CSC as a tumor cell that has the potential to generate a full tumor implies that all CSCs need to be killed to reach a permanent local tumor control (3, 4). Determination of permanent local tumor control as an endpoint of experimental or clinical studies is currently

the only available functional assay of CSC survival after irradiation (3, 4). Assuming a linear increase of the absolute number of CSCs with increasing tumor volume, the known negative correlation of the tumor-control probability with the logarithm of the tumor volume indicates that the number of CSCs that need to be inactivated by irradiation is an important determinant of local tumor control (Fig. 1; refs. 5–7). This interrelation is supported by seminal experiments showing a correlation between transplantability of different tumor models and the tumor-control dose 50% (TCD50) after single dose irradiation (8). The fact that this correlation still exists when fractionated radiotherapy is used in the clinical setting, in which several radiobiological parameters and intertumoral heterogeneity affect the results, implies that the number of CSCs is among the dominant predictors of tumor cure after radiotherapy. This finding is supported by a close correlation of TCD50 after fractionated irradiation over 6 weeks with TCD50, after single dose under clamp hypoxia, in which 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 tumor radiocurability (10).

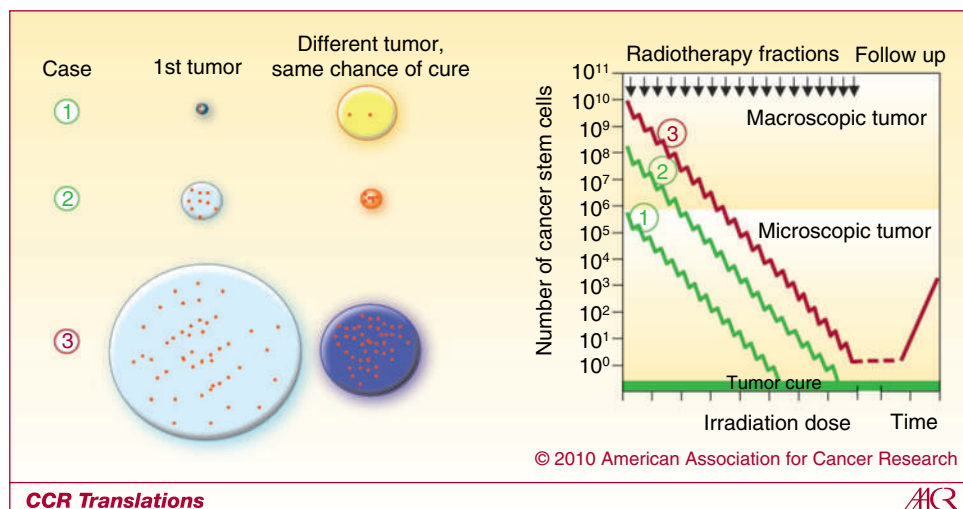
Although the importance of the number of CSCs for local tumor control is obvious, until recently no marker was available that could measure the stem cell density of different tumors in the clinics. Such markers would have important potential as predictors for local tumor control. De Jong and colleagues, for the first time, show, in a group of patients with comparable tumor stages and treatments, that CD44 mRNA expression, as well as CD44 immunohistochemical score, was the only significant predictor for local tumor 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, in which 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

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**Fig. 1.** In the same individual tumor, the CSC number is expected to increase linearly with tumor volume. A higher number of CSCs requires a higher irradiation dose to permanently cure the tumor. Each irradiation fraction kills a certain percentage of the CSCs, whereas between the fractions, the surviving CSCs can repopulate and increase their number. The figure shows three different cases. In case 1, the first tumor (light blue) is small and has a given number of CSCs. Alternatively, another tumor (yellow) may have a larger size but lower CSC density, and thus, the same low absolute number of CSCs. Both tumors will be cured already after a relatively low irradiation dose (right panel). In case 2, the first tumor (light blue) is irradiated at a 100 fold larger volume. The CSC density is still the same, whereas the absolute number is increased. The irradiation dose needed to cure this tumor is higher because of the higher number of CSCs. The same dose would be needed for another tumor (orange) of a smaller size, but the same absolute number of CSCs. In case 3, the volume and the number of CSCs in the first tumor (light blue) have again increased 100 fold before the start of radiotherapy. Here, the applied irradiation dose is not sufficient for permanent local tumor control, because one CSC survived. The same would apply for a smaller tumor (dark blue) with higher CSC density.

cancer cell lines were evaluated. Here, the CD44 expression correlated with *in vitro* plating efficiency, a marker for the percentage of clonogenic tumor cells, but not with intrinsic radiosensitivity of the clonogenic tumor cells *in vitro*. Although clonogenic cells *in vitro* do not necessarily reflect CSCs *in vivo* (4), these data support that CD44 correlates with the number and not with the intrinsic radiosensitivity of CSCs.

The data published by de Jong and colleagues are of great relevance for translational research in radiotherapy. For the first time, a clinical dataset on a CSC-related biomarker has become available, which is consistent with the preclinical experiments showing the importance of intertumoral heterogeneity of CSC density for local tumor control after radiotherapy. On the basis of these results, CD44 should be further evaluated in patients with early laryngeal cancer for its potential as a predictive biomarker for individualized treatments; for example, radiation dose escalation or primary use of surgery in those tumors 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 tumor volumetry might permit estimating the absolute CSC number of tumors. Radiobiologically, a direct correlation is expected between the logarithm of CSC number and the dose necessary for tumor control. If the CSC number were available for a given tumor, in principle, this parameter could be directly integrated into dose prescription and radiation treatment planning.

Another important avenue for further research is validating 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 that determine outcome. One, therefore, might speculate that CD44 may lose some of its dominance as a predictor and may assume clinically relevant potential as a 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 markers that may be used for the accumulation of CSCs, thereby increasing understanding of CSC biology. The study by de Jong and colleagues intelligently combines such technologies with analysis of local tumor control, the only clinical endpoint that is specific for CSC survival after radiotherapy. This study, 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.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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