International Journal of
Radiation Oncology
biology • physics

www.redjournal.org

Genomics Reloaded: Rise of the Expression Profiles

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Received Oct 11, 2017. Accepted for publication Oct 12, 2017

In this issue of Oncology Scan, similar to previous head and neck cancer Oncology Scans (1-7), we examine the use of genomic signatures to predict response to head and neck cancer therapy (8-10). In the past decade and a half, multiple teams have identified and developed gene expression profile signatures capable of subclassifying head and neck cancer, prognosticating outcome, and predicting treatment response. We discuss the implications of these genomic signatures for head and neck cancer patients, how these signatures can be applied to other therapies, their use to assist in preclinical studies, and suggestions on how this work can move the field forward, not just for head and neck cancer but potentially for all types of cancers.

Klinghammer et al. Basal subtype is predictive for response to cetuximab treatment in patient-derived xenografts of squamous cell head and neck cancer. *Int J Cancer* 2017. (9)

Summary: This translational genomics article hypothesized that basal subtype tumors (which are known to have epidermal growth factor receptor [EGFR] pathway activation) would be preferentially sensitive to anti-EGFR therapy. To test response to common chemotherapies (eg, carboplatin, cisplatin, or cetuximab), the authors used patient-derived xenografts (PDXs). Patient-derived xenografts are a powerful preclinical model system in which fragments of a patient tumor are engrafted onto an immunocompromised mouse to test novel therapeutics or investigate tumor biology (11, 12). Tumors were harvested 3 weeks after initial treatment and analyzed using next-generation sequencing to assess for mutational differences between tumors and using gene expression microarrays. They performed a number of analyses (13-15) to classify the PDXs into 1 of 3 subtypes (ie, basal, classic, and mesenchymal/inflamed). The only correlation between treatment and subtype was seen for cetuximab treatment. Tumors of the basal subtype, enriched for the EGFR pathway, responded favorably to cetuximab. Tumors of the mesenchymal/inflamed subtype demonstrated negative enrichment for the EGFR pathway and did not respond to cetuximab. Mutational analysis demonstrated no significant differences between subtypes, but among the most frequently altered genes overall were *TP53* and *PI3KCA*, consistent with previously published work (16, 17). Although the authors noted enrichment in mutated *PI3KCA* within the mesenchymal/inflamed subtype, no association between systemic therapy and mutational status was observed, consistent with other studies (18).

R.J.K. was supported in part by a University of Wisconsin Carbone Cancer Center Support Grant (P30 CA014520) and a Wisconsin Head and Neck SPORE Grant (P50 DE026787).

Bossi et al. Functional genomics uncover the biology behind the responsiveness of head and neck squamous cell cancer patients to cetuximab. *Clin Cancer Res* 2016. (8)

Summary: In this retrospective genomic analysis study, patients treated with chemotherapy and cetuximab between 2008 and 2012 were identified. A total of 40 patients with recurrent or metastatic head and neck squamous cell carcinoma were included in the analysis. Patients were stratified into 2 groups: long progression-free survival (PFS) (n = 14) or short PFS (n = 26). Whole-genome gene expression profiling was performed on formalin-fixed, paraffin-embedded tumor specimens. Importantly, the authors rigorously assessed their dataset using modeling-prediction software, existing validation datasets, and technical validation for gene expression. A gene signature based on more than 500 differentially expressed genes was independently validated against a metastatic colorectal

cancer dataset from patients treated with cetuximab. The identified signature was able to demonstrate a significant difference between short and long PFS in the colorectal cancer dataset, suggesting a robust signature with relevance across tumor types. Further stratification of the dataset into molecular subtypes (see above) demonstrated that the long-PFS group most closely resembled the basal subtype, consistent with the work of Klinghammer et al. Bossi et al also demonstrated upregulation of the RAS pathway, particularly oncogenic *KRAS* mutants, within the short-PFS signature, consistent with studies in other types of cancers in which RAS activation correlates with cetuximab resistance (19, 20). They validated these findings in head and neck squamous cell carcinoma cell lines that were either sensitive or resistant to cetuximab treatment.

Scott et al. A genome-based model for adjusting radiotherapy dose (GARD): A retrospective, cohort-based study. *Lancet Oncol* 2016. (10)

This large-scale retrospective study introduced the concept of genome-based adjustment of radiation therapy dose (GARD) using patient gene expression profiles to help predict an optimal radiation dose; GARD was derived using a previously established gene expression—based radiation sensitivity index and the linear-quadratic model. Using primary tumor samples prospectively collected through the Total Cancer Care protocol, this study drew from more than 8000 tissue samples representing 20 different tumor types that underwent gene expression analysis. According to their stratified gene expression profile, tissue groups were assigned a GARD score based on their sensitivity to 3 different radiation dose ranges: 45, 60, or 70 Gy and higher. Patients with high GARD scores exhibited improved distant metastasis—free survival rates, and GARD remained an independent prognostic (and predictive) factor after controlling for known variables. The authors tested GARD against 5 different publically available expression profiles, and for each dataset GARD remained highly predictive of local control and relapse-free and overall survival (10). In particular, samples drawn from the Total Cancer Care protocol demonstrated that cervical and oropharyngeal squamous cell carcinoma patients had a high GARD score, whereas non-oropharyngeal head and neck cancer was associated with a lower GARD score.

Comments: Therapies targeting EGFR represented the first "breakthrough" molecularly targeted drugs for head and neck cancer. In randomized studies, the addition of cetuximab to radiation (21) or systemic chemotherapy (22) improved outcomes. There remains significant uncertainty as to how to use these drugs to deliver personalized treatment to head and neck cancer patients—the right therapy to the right patient at the right time. Simple approaches, such as stratifying patients by high versus low EGFR status, have failed to demonstrate any correlation with outcome and cetuximab use (23).

The first 2 articles presented here demonstrate the potential power of genomic data to predict responses to anticancer drugs. Klinghammer et al demonstrated through molecular subtyping of their head and neck cancer PDXs that they can predict response to cetuximab therapy and that a favorable response was strongly correlated with basal subtype. Bossi et al developed a gene expression profile that could stratify cetuximab-treated patients into long and short PFS (19 vs 3 months, respectively). Together, these studies suggest that we may be able to identify a group of patients most likely to benefit from the use of cetuximab and illustrate how the use of preclinical model systems can enable us to identify predictors of therapy response. Given the significant cost of prospective therapeutic trials and the critical metric of improving patient overall survival, the need to design rational, data-driven clinical studies is paramount. We hope that the use of preclinical models such as these could be used to inform patient selection criteria for future clinical trials. Had this approach been used for trials such as Radiation Therapy Oncology Group protocol 0522, perhaps the results of the study would have been different, because investigators could have identified the group of patients most likely to benefit from a novel therapy. To be sure, genomic analyses must be tested in prospective trials to demonstrate their true value in cancer care. These studies also demonstrate ways in which powerful preclinical model systems can be used to move the field forward.

In the third article, the group of Javier Torres-Roca sought to use their radiation sensitivity index to personalize radiation dose using GARD (10, 24). This article strongly suggests that precision medicine, traditionally associated with medical oncology and systemic therapy, may yet find an important role in radiation oncology. Although radiation oncologists have long personalized radiation fields to individual patients, we have largely left the radiation dose used to treat a given tumor fixed. The use of GARD provides one example of how we could identify a group of patients who will respond to radiation treatment, and it may also enable us to identify the optimal dose for each individual patient's cancer. Clearly the radiation sensitivity index and GARD will require further evaluation in prospective trials to validate our ability to continue to cure cancer patients while personalizing the radiation dose they receive.

It is easy to envision a day when gene expression profiles will be used to personalize therapy for our patients. These gene expression profiles are not the end but rather a new beginning and will serve as a jump point for clinicians and scientists to make additional discoveries that will continue to advance the field of medicine. Radiation has been used to cure cancer patients for more than a century. By embracing the personalization of cancer care, we will continue to provide valuable, effective care for the next 100 years.

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