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Mini-review

Comprehensive molecular tumor profiling in radiation oncology: How it could be used for precision medicine

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ABSTRACT

New technologies enabling the analysis of various molecules, including DNA, RNA, proteins and small metabolites, can aid in understanding the complex molecular processes in cancer cells. In particular, for the use of novel targeted therapeutics, elucidation of the mechanisms leading to cell death or survival is crucial to eliminate tumor resistance and optimize therapeutic efficacy. While some techniques, such as genomic analysis for identifying specific gene mutations or epigenetic testing of promoter methylation, are already in clinical use, other "omics-based" assays are still evolving. Here, we provide an overview of the current status of molecular profiling methods, including promising research strategies, as well as possible challenges, and their emerging role in radiation oncology.

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Introduction

During the last decade, the outcome for cancer patients receiving radiotherapy or chemoradiation has continuously improved. This success is not only due to technical and imaging advances and the more accurate delivery of radiation to a tumor, but also to the implementation of molecular therapeutics into radiation oncology treatment regimens, which allows for more specific cancer cell targeting [1,2]. Although very encouraging results have been obtained in a subset of patients with specific molecular-targeted drugs, multiple clinical studies indicate that tumor heterogeneity is a major obstacle resulting in varied tumor responses, including non-response, to targeted therapy [3]. The molecular and phenotypic heterogeneity is present prior to treatment and the treatment itself can select for resistant subpopulations and induce further heterogeneity leading to tumor cell resistance. Thus, elucidation of the complex molecular processes and identification of potential de novo and bypass signaling can contribute to the optimization and individualization of patient therapy [4,5]. Exploiting the tumor phenotype before treatment as well as the adaptation of tumor cells to the changes that result from therapy is a novel approach to effective precision cancer treatment. An interest of our laboratory is understanding how cancer and normal cells adapt to radiation and how these phenotypic changes might be used to enhance the efficacy of radiotherapy [6–8]. With increasing knowledge about molecular mechanisms, it is becoming more evident that the effect of radiotherapy on tumor cell survival is not only dependent on physical beam properties, radiation dose, and DNA damage but is also strongly influenced by radiation-induced perturbation of biological processes, a concept named "focused biology" [9]. This implies the potential use of radiation in a novel way in combination with both molecular targeted drugs and also immunotherapy [10,11].

In addition to the targeting of molecules expressed in cancer cells, the therapeutic potential of immune response modulation is currently under intense evaluation in clinical trials [12–14]. This approach is based on the observation that some tumors have the ability to suppress the antigen-induced activation of leukocytes resulting in reduced cancer cell killing and poor patient survival [15–17]. Therefore, immune checkpoint inhibitors such as ipilimumab and nivolumab, which modify the interaction between the tumor cells and T lymphocytes, can be used to abrogate the tumor-mediated immune inhibition [17]. First results in patients with melanoma and advanced non-small cell lung cancer (NSCLC) are very promising [12,13], although a recent randomized phase III trial in patients with metastatic prostate cancer showed no significant survival benefit of ipilimumab treatment after radiotherapy compared with the placebo group [18]. Future studies will clarify the role of these compounds in radiation oncology.

The development of methods facilitating the simultaneous analysis of multiple molecular characteristics in a small tumor sample was a precondition for omics-based assays. With the implementation of DNA microarrays into cancer research, it was possible for the first time to determine the expression of thousands of genes in one assay and detect disease- or resistance-driving mutations in

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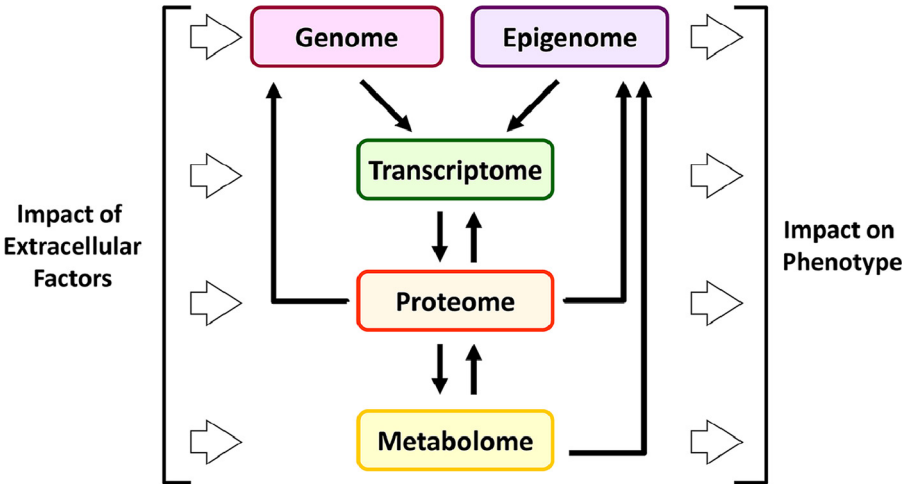

Fig. 1. Schematic representation of how genome, epigenome, transcriptome, proteome and metabolome modulate each other and their impact on the cellular phenotype. The DNA (genome) and the epigenetic modifications (epigenome) regulate transcription of RNA (transcriptome). The mRNA is translated into proteins (proteome). These proteins including enzymes modulate the expression of metabolites (metabolome), but also transcription, genetic and epigenetic markers. Extracellular factors like irradiation or chemotherapy can affect all molecular processes, with the strongest influence at the metabolic level. The cellular phenotype is substantially determined by all molecules.

tumor tissue on a large scale [19,20]. Next-generation sequencing enabled the analysis of a complete human genome in one day, a process, which took several years in the past [21,22]. The tremendous technical and methodical advances in the last two decades and the possibility to apply these assays in a high-throughput setting have greatly contributed to the clinical and scientific significance of omics-based methods.

The family of "omics" is growing including analysis of gene mutation status and RNA expression [3,23–26], epigenetic changes such as promoter methylation and histone modifications [27,28], protein expression and phosphorylation [29–31], and metabolite levels [32,33], all of which can affect the radiation and treatment response of tumors (Fig. 1). Extensive omics-based analysis of cancer cells before, during and after radio- and chemotherapy could be used to reveal molecular mechanisms, predict therapy efficacy and guide therapy as the tumor adapts to treatment. In this review, we summarize and discuss key findings of genomic, transcriptomic, proteomic, epigenomic and metabolomic studies and the role of the different molecular profiling methods in radiation oncology.

Genomic analysis and its potential for patient stratification

Given that both treatment sensitivity to a molecular compound as well as intrinsic or acquired resistance of tumor cells can be caused by gene mutations, genetic analysis is considered to be crucial for choosing the most effective therapy [34,35]. While matching the "right" drug to a mutation is a major area of research, the efficacy of the new molecular therapeutics is very sensitive to structural changes in the target molecule or the functional changes in the downstream signaling pathway and therefore the examination of gene mutation status prior to treatment is essential [36–38].

Inhibitors of the epidermal growth factor receptor (EGFR) were among the first targeted therapeutics whose treatment outcome could be linked to a specific genetic profile including the molecule being targeted as well as downstream pathways, as discussed below. In combination with radiotherapy, clinical studies with the EGFR antibody cetuximab showed promising results in patients with head and neck squamous cell carcinoma (HNSCC), resulting in the approval and implementation of this drug in clinical treatment regimens [39,40]. However, cetuximab failed to improve the overall outcome for patients with colorectal tumors [41,42] and NSCLC [43]. Extensive genomic studies show that one potential factor for the effect of inhibitory EGFR antibodies was the mutation status of the *Kirsten rat sarcoma 2 viral oncogene homolog* (*KRAS*) and *v-Raf murine sarcoma viral oncogene homolog B* (*BRAF*) genes, coding for two signaling molecules of the EGFR pathway [37,44–47]. Tumors expressing wildtype *KRAS* and *BRAF* had a significantly higher control rate when cetuximab therapy was applied, while the presence of specific *KRAS* or *BRAF* mutations diminished the tumor response [37,44,45]. In patients with *BRAF*/*KRAS* wildtype rectal carcinoma, receiving neoadjuvant radio-chemotherapy, cetuximab increased overall survival and radiologic tumor response rate, while there was no significant effect in the whole patient population (including patients with both wildtype and mutated *BRAF*/*KRAS* tumors) [48]. However, factors other than the genetic background may also be important for the therapeutic efficacy of EGFR antibodies, as recent studies show that even in *BRAF* or *KRAS* wildtype colorectal carcinoma the response to cetuximab is not invariably present [41,42,49,50].

Similar to EGFR antibodies, EGFR tyrosine kinase inhibitors have been found to be more effective in a specific subset of patients. After the first generation EGFR inhibitor gefitinib was approved for treatment of NSCLC in 2003, two clinical studies showed no significant survival benefit, which led to the use of the drug being restricted to patients who previously benefited from gefitinib without understanding the reasons for the differential clinical responses [51]. Later, sub-analyses revealed that patients with specific activating mutations in the EGFR kinase domain had a much better response rate [3,52]. Similar findings were observed in clinical trials with the EGFR inhibitors erlotinib and afatinib [38,53]. Several recent phase II studies describe promising results for the use of EGFR kinase inhibitors in combination with radiotherapy [54–56]. In these trials, when erlotinib was added to the treatment regimen, the outcome and response in patients with advanced stage NSCLC were better than the results from published studies [54–56]. Interestingly, Komaki et al. did not find a correlation between EGFR mutation status and response [54], although this may be due to the relatively small patient numbers or to different molecular mechanisms with the drug used alone or in combination with radiation. Therefore, further studies are needed to clarify the factors modulating the efficacy of EGFR kinase inhibitors combined with radiotherapy and radiochemotherapy. Within the focused biology concept, the therapeutic effect of drugs that have been developed as mono-therapeutic agents may be improved by using them with radiation, for example, when the target expression is upregulated by radiotherapy.