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# 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.

### Keywords

molecular profiling; precision medicine; radiation oncology; targeted therapy

## 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

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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 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 promotor 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 (Figure 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 was better than the results from published studies [54–56]. Interestingly, Komaki and colleagues 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 radio-chemotherapy. 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.

Genomic analysis can also be used to personalize treatment and enhance the efficacy of drugs on malignant tissue by exploiting existing differences in the structure of target molecules [35]. For example, the BRAF inhibitors vemurafenib and dabrafenib are designed to inhibit only BRAF with a substitution of the amino acid valine at position 600 with glutamic acid (V600E), but not wildtype BRAF [35]. This V600E mutation is frequently found in melanoma and papillary thyroid carcinoma [57]. Inhibition of BRAF results in a significant increase of overall and disease-free survival in patients with BRAF-mutant melanoma [35]. Despite these promising results, the majority of tumors develop resistance over time, leading to disease recurrence and progression [58]. Genomic analysis revealed a variety of underlying mechanisms including changes of the BRAF sequence, itself, and activating mutations of downstream targets [36,58].

To date, there are few clinical studies analyzing the efficacy of BRAF inhibition in combination with radiotherapy. Satzger and colleagues reported several cases of severe radiation dermatitis in patients with metastatic melanoma treated concomitantly with dabrafenib or vemurafenib [59]. Interestingly, all tumors showed no response to the treatment, indicating a radiosensitizing effect on normal tissue with wildtype BRAF, but not on malignant cells harboring BRAF V600E mutations. One possible explanation for this finding is that the effect of radiation on the skin is potentiated by the drug-related cutaneous side effects of BRAF inhibitors including erythema and hand-foot syndrome which are thought to be caused by a paradoxical activation of the mitogen-activated protein kinase (MAPK) pathway in wildtype BRAF cells [59–61]. This further emphasizes how molecular targeted drugs can have different effects when they are used in combination with radiation compared to mono-therapeutic application.

In addition to activating mutations, genetic changes such as gene deletions can be important for the efficacy of targeted therapy. Inhibition of the poly (ADP-ribose) polymerase (PARP), an enzyme involved in repairing DNA single strand breaks (SSBs), has no radiosensitizing effect in normal cells due to an efficient repair of SSBs by homologous recombination (HR). In cells with a defect in HR (e.g. by a frameshift mutation in the *breast cancer, early onset [BRCA] 1* or *BRCA2* gene), PARP inhibitors strongly reduce cellular radiation survival [62]. As hereditary *BRCA* mutations promote the development of breast and ovarian cancer, a certain percentage of these tumors are BRCA1 or BRCA2 negative. These tumors have been successfully treated with PARP inhibitors, such as olaparib [63,64]. However, in most clinical trials PARP inhibitors were predominantly combined with cytotoxic drugs and not

with radiotherapy, so that the role of PARP inhibition in radiation therapy is not yet clearly defined.

Although these data show that genomic analysis contributes to the optimization of targeted therapy, many resistance mechanisms are based on cellular processes like transcription or protein signaling and not solely on genetic alterations. Therefore, further molecular profiling techniques are required to evaluate the tumor response in more detail.

## The use of epigenomics for predicting outcome and drug efficacy

Epigenetic alterations play an important role not only in many physiologic processes including DNA replication and repair, but also in disease development and cancer cell resistance to therapy [65,66]. DNA methylation can regulate promoter activity and therefore affect gene expression and silencing [65]. Additionally, histone modifications at specific residues are involved in gene activation or inactivation and have been shown to modulate radiosensitivity [27,28,66–68].

One example of the use of epigenetic characteristics as a predictive marker for cancer therapy is the promoter methylation status of the *O6-methylguanine methyltransferase* gene (*MGMT*) in glioblastoma. Here, a highly methylated and, therefore, silenced promoter correlates with a better outcome for patients after radiation and treatment with temozolomide, an alkylating cytotoxic drug [69,70]. In esophageal cancer, decreased DNA methylation of nine selected genes was found in tumors responding to combined radio- and chemotherapy compared to non-responders [71]. The identified genes included *MGMT*, cell cycle regulators *p16* and *p57* and *runt-related transcription factor 3* (*RUNX-3*) [71]. In addition, a recent study demonstrated a significant association between esophageal carcinoma response to definitive chemoradiation and promoter methylation of the *zinc finger protein 695* (*ZNF695*) gene coding for a protein with a not yet well characterized function [72]. Siegel and colleagues examined the DNA methylation status of patients with locally advanced anal carcinoma prior to radiochemotherapy. Patients were stratified into a low risk or a high risk group on the basis of clinical parameters including tumor size and lymph node involvement. The high risk group, which had a significantly reduced overall and disease-free survival, had an increased promotor methylation of seven genes compared to the low risk group [73]. Because most of these studies evaluated the epigenetic modifications only before treatment, changes due to radiation or chemotherapy, which might inform how to adapt treatment based on tumor response, were not taken into account.

Analysis of breast cancer biopsies taken before radiotherapy and after exposure to 10 – 24 Gy showed that the DNA methylation status of genes involved in the immune response could be modulated by irradiation [74]. While DNA methylation in five genes prior to radiotherapy and in six genes after irradiation could be identified as prognostic markers, only two genes were present in both groups, indicating high radiation-induced variability [74]. So, much remains to be done to examine the impact of methylation status on cellular function.

Like promoter methylation, specific histone modifications have also been linked to treatment outcome and tumor response of patients with different cancer types [75–77]. In pancreatic

carcinoma, high levels of dimethylated (H3K4me2) or acetylated histone 3 (H3K18ac) are associated with better survival after adjuvant radiochemotherapy, independent of other clinical factors [75]. Similarly, H3K4me2 expression in prostate cancer or NSCLC was low in patients with poor overall and disease-free survival, indicating that histone methylation might be a promising prognostic marker in different tumor entities [76,77].

## The use of transcriptomic data to clarify molecular processes and to find suitable drug targets

In contrast to genomic analysis, which is more stable and therefore mainly used to assess the mutational status of cancer cells prior to therapy or when the tumor becomes resistant, gene transcription is a highly dynamic process. To determine the mRNA expression levels of targets at the beginning of the treatment and during therapy is critical for precision medicine. The transcriptome is divided into coding and non-coding RNA. Coding RNA is translated into proteins, while non-coding RNA, including micro RNA (miRNA), long non-coding RNA (lncRNA) and others, is considered to have a regulatory function [78,79]. As non-coding RNA affects the sensitivity to ionizing radiation or chemotherapy, it can also serve as a potential drug target to increase therapy response. [24,80–84].

Both coding and non-coding RNA expression levels have been analyzed in tumors to predict treatment response and clinical outcome [85–87]. Wong and colleagues examined the mRNA expression profiles of patients with cervical cancer prior to radiotherapy to find differences between radiosensitive and radioresistant tumors. Patient stratification into each group was based on clinical outcome and survival time. In radioresistant cancer samples for example, several genes coding for transcription factors, immune modulators or proteins involved in cytoskeletal organization were upregulated [85].

In patients with pancreatic cancer receiving adjuvant chemotherapy or chemoradiation after surgery, low expression of the miRNA, miR-21, was shown to be a negative prognostic marker in tumor tissue [86].

As RNA levels can change upon exposure to ionizing radiation or chemotherapy, analysis of the transcriptome during and after treatment is essential to understand resistance mechanisms and monitor expression of possible therapeutic targets [6,7,10]. A study in patients with rectal carcinoma examining the effects of radio-chemotherapy on mRNA levels in normal and malignant tissue showed a differential expression of genes involved in cell adhesion and leukocyte migration [87]. In line with these results, a specific gene cluster, including cell adhesion proteins and molecules involved in apoptosis is upregulated in cervical cancer after chemoradiation, while expression of cell cycle regulators is reduced [88]. Changes in transcription after treatment can also provide information about the therapeutic efficacy of molecular compounds. The clinical response to cetuximab and chemoradiation on gene expression was evaluated in patients with rectal carcinoma [89]. The authors observed a prolonged disease-free survival when EGFR was upregulated after treatment.

Few studies examined the expression of lncRNA in cancer samples [90,91]. Prensner and colleagues found an increased level of the lncRNA SChLAP1 in metastatic prostate cancer compared to patients with localized disease. Additionally, a high SChLAP1 expression was an independent negative predictive marker and correlated with reduced overall and disease-free survival [90]. In NSCLC, low levels of the lncRNA SPRY4-IT1 are associated with advanced tumor stage, lymph node infiltration and poor prognosis, while overexpression of SPRY4-IT1 *in vitro* leads to growth arrest and apoptosis [91]. The exact molecular mechanisms of lncRNA in the regulation of cell death and survival, however, are not yet fully understood.

Monitoring the expression of molecular targets during therapy is another important application of transcriptomic analysis. On the one hand, as transcription can be modulated by radiation, downregulation of the target could attenuate the efficacy of molecular drugs. On the other hand, radiotherapy might be used to enhance the expression of specific molecules and enabling in this way innovative molecular targeting approaches. When analyzing the transcriptome in tumor cells and biopsies to identify new promising targets for radiation oncology, both the radiation dose and the fractionation regimen have to be taken into account as these factors can strongly influence RNA expression [6,7,10].

In summary, transcriptomic analysis is an important tool for the examination of molecular processes in cancer cells. The assays can be performed with small tumor samples, for example, biopsies and potentially circulating cancer cells in the blood, and are relatively cost and time efficient. These techniques, therefore, have great potential for optimizing personalized cancer therapy.

## Proteome and phosphoproteome analysis in revealing bypass and resistance mechanisms

Expression and functionality of target molecules have a critical impact on the efficacy of molecular therapeutics. In particular, the effect of kinase inhibitors can be modulated by the enzymatic activity status of the target, which is often controlled by post-translational modifications, such as protein phosphorylation. As mRNA levels do not necessarily correlate with protein expression due to other regulatory processes, including translation and protein degradation, proteomic and phosphoproteomic profiling of cancer cells can give valuable information about the complex cellular signaling network [92].

A recent study showed that specific phosphoproteins were upregulated in HNSCC tumors compared to normal mucosa [93]. The activated pathways included checkpoint signaling, regulators of translation and MAPK-associated molecules. Reduced phosphorylation of ErbB3 was found in undifferentiated tumors, while perineural invasion and lymph node metastasis, both negative prognostic markers, significantly correlated with low phosphorylated extracellular signal-regulated kinase (ERK) levels [93].

Rikova and colleagues examined the phosphoproteome in NSCLC patient samples and found different activation clusters of tyrosine kinases [94]. Some tumors expressed only one or two highly active kinases, while other tumors showed activated focal adhesion- or growth

factor receptor-related signaling [94]. A correlation analysis with clinical parameters including treatment outcome or therapy resistance was not performed. In rectal carcinoma, phosphorylation of beta-Catenin and Chk2 was shown to be enhanced in tumors with a good response to neoadjuvant chemoradiation, while the GSK3beta phosphorylation level was decreased [95]. The authors only examined phosphorylation before therapy and did not take changes in phosphorylation into account, which occurred during treatment.

Despite the great potential of phosphoproteomic techniques for clarification of molecular processes, several limitations restrain their clinical application, especially in serial examinations. One critical factor is that, in comparison to RNA analysis, many proteomic assays require a relatively large amount of tissue to obtain valid results. Because sample sizes are often limited, as in the case of tumor biopsies, the extended application of proteomic analysis can be very challenging [92]. Moreover, protein phosphorylation is known to be volatile and susceptible to external factors such as temperature or mechanical stress. Therefore, differences in sample processing can compromise the results.

While the aforementioned factors impede the use of phosphoproteomic and proteomic profiling in the clinic, it has undoubtedly an important role in preclinical work. Several studies highlight the importance of the analysis of protein phosphorylation in elucidating the radio- and chemoresistance mechanisms and identifying promising drug combinations [6,30,31,96–103]. For example, a phosphoproteome array in HNSCC cells treated with cetuximab showed activation of the c-Jun N-terminal kinase (JNK) pathway which attenuated the efficacy of cetuximab itself [30]. In line with these findings, combined inhibition of EGFR and JNK significantly reduces radiation survival of HNSCC cells suggesting a promising approach to overcome drug resistance [30]. Similarly, targeting of beta1 integrin leads to enhanced phosphorylation of EGFR/MAPK associated signaling [31]. Dual inhibition of EGFR and beta1 integrin can significantly increase *in vitro* and *in vivo* cellular radiosensitivity, indicating the existence of molecular bypass mechanisms in cancer cells to avoid cell death after targeted therapy [31,100]. Phosphoproteomic analysis of human epidermal growth factor receptor 2 (HER2)-positive breast cancer cells showed that trastuzumab resistance could be promoted by activation of different prosurvival pathways including focal adhesion signaling and growth factor receptor-associated pathways. An siRNA-mediated knockdown of these activated proteins restored sensitivity to HER2 inhibition [103].

Although these data are highly interesting for clarifying the underlying resistance mechanisms, further studies in a broad patient population are needed to validate the clinical relevance of these results.

## The emerging role of metabolomics in radiation oncology

Metabolism in tumor cells is often different from that in normal tissue. On the one hand this can be caused by intrinsic factors like mutational alterations of important enzymes or differential expression of molecules [33]; on the other hand, also extracellular factors, for example the tumor microenvironment, can have a critical impact on metabolic pathways and metabolite levels. Moreover, drug therapy and systemic diseases, like diabetes or hepatic

failure, can affect the results of metabolomics assays. This susceptibility to interference further complicates the use of these techniques in the clinic. Nevertheless, integrative analysis of the tumor metabolome is indispensable for understanding the cellular phenotype and mechanisms of cancer cell resistance (Figure 1).

One example regarding the impact of cellular metabolite levels on tumor formation and progression is the mutation of the isocitrate dehydrogenase (IDH) [104]. IDH mutations found in glioma, chondrosarcoma and hematologic malignancies result in the accumulation of D-2-hydroxyglutarate which inhibits several enzymatic reactions involved in epigenetic modifications and cell signaling [105–108]. As the IDH mutation status is a strong predictor for patient outcome, non-invasive imaging of D-2-hydroxyglutarate with nuclear magnetic resonance spectroscopy could be used for risk stratification when genetic tumor profiling is not feasible [104,109–111]. Additionally, the development of therapeutics targeting only mutated IDH might be a promising approach for treatment of these tumors.

Recent studies have shown that metabolomic profiles can be used as prognostic markers in some cancers and also helps to identify new molecular targets [112–114]. In colorectal cancer patients, a specific expression pattern of 15 metabolites including lactate, cysteine and palmitoleate correlates with overall survival and tumor recurrence after surgery and chemotherapy [115]. In line with these results, the metabolome in breast cancer patients was found to be different in biopsies, based on prognostic criteria, like hormone receptor status, lymph node involvement and tumor stage [116,117]. Wibom and colleagues measured the extracellular level of multiple metabolites in glioblastoma and adjacent brain tissue in patients before and during radiotherapy using microdialysis catheter. While the glucose concentration was lower in the tumor compared with the surrounding normal tissue, the levels of several essential amino acids were increased [32]. After radiation, some metabolites, like alanine and inositol, were upregulated solely in malignant tissue. In contrast, normal brain tissue showed radiation-induced expression of arabitol and pentonic acid.

Although blood samples of cancer patients have also been used to analyze the metabolic changes during cancer therapy, there is a wide variation, even in healthy subjects, making the data interpretation more challenging [118,119]. Despite this variability, an increase in serum octanoylcarnitine and decanoylcarnitine levels during and after radiochemotherapy was observed in patients with esophageal cancer who responded to treatment [118]. Similarly, patients with nasopharyngeal carcinoma were divided into prognostic groups based on the metabolic profile of serum samples during radiotherapy [119].

Overall, evaluation of the metabolome is a novel, promising field. Elucidation of metabolic processes in cancer cells after treatment can help to identify resistance mechanisms and promising targets for molecular therapy.

## Conclusion

Omics-based technologies greatly improved our understanding of the complex molecular mechanisms in cancer cells (Figure 2). The discovery of cancer-driving mutations enabled

the development of molecular targeted drugs, while the expression profiles of RNA and proteins have already been used to predict treatment outcome and identify patients with high risk for treatment failure. Additionally, understanding the functional abnormalities in the pathways could be used for treatment selection and understanding adaptation and survival of cancer cells during and after therapy.

Some challenges exist, which complicate the clinical use of these new techniques. One major factor is intra-tumoral heterogeneity, with a range of differences in the expression of RNA, proteins and metabolites among sub-populations within the tumor. Consequently, in the evaluation of prognostic molecular profiles, this heterogeneity within one tumor can obscure potential correlations with survival or disease progression. Moreover, because expression patterns can be modulated by multiple external factors, patient comorbidities and the therapy itself, repetitive examinations are required to understand tumor response and adaptation. In recent years, circulating cancer cells or cell-free tumor DNA in the blood have been used for molecular profiling as so-called liquid biopsies [120,121]. These techniques have some advantages compared to conventional tissue biopsies. They are minimal-invasive and allow immediate detection of changes in cancer cells during treatment. Additionally, there is no need for tissue preservation such as formalin fixation, which modifies the DNA structure and therefore can interfere with correct genotyping of cancer cells [122]. Although several studies particularly in patients with advanced disease successfully implemented liquid biopsies for monitoring therapy resistance [123–126], some technical limitations can hinder the broad use in the clinic. To date, there is no standardized procedure for extraction and enrichment of tumor material from the blood. Moreover, the number of circulating cancer cells or DNA fragments can be very low in non-metastatic patients making the molecular analysis challenging [121]. There is also some uncertainty in whether malignant cells from all metastatic or primary sites are equally represented in the circulating fraction [121]. Nevertheless, liquid biopsies have a great potential, especially for cancer genomics and optimization of mutation-based tumor therapy.

Another important challenge for integrating omics-based methods in standard patient care is the large amount of data, demanding sophisticated evaluation techniques and integrative concepts [127]. There are new trial designs from NCI based on targeting particular mutations (Molecular Analysis for Therapy Choice [NCI-MATCH]) although it is likely that there will be confounding factors within the cell and its environment that impact response beyond the presence of a specific mutation. Appropriately sized randomized (or at least stratified) trials with appropriate biomarkers are warranted to show the clinical relevance of the laboratory data.

Given that radiation can induce an adaptive response which potentially modifies heterogeneity and affects drug efficacy, the role of radiation perhaps with new fractionation schedules, along with molecular-targeted and radio-chemotherapy may have an important impact on cancer cure as well as the potential for repurposing drugs already used in the clinical setting. Fortunately, with diagnostic technology and data processing speed increasingly becoming better and more affordable, the goal of precision medicine based on extensive molecular tumor profiling seems to be within reach, employing novel “big-data”

analysis techniques, hypothesis-based trials and mechanism-based assessments of the results.

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## Highlights

- Tumor cell resistance to chemoradiation and targeted drugs affects patient outcome
- Novel techniques can be used to stratify patients and predict therapy efficacy
- Radiation can modulate target expression and improve efficacy of targeted therapy
- Development of personalized treatment strategies enables precision medicine

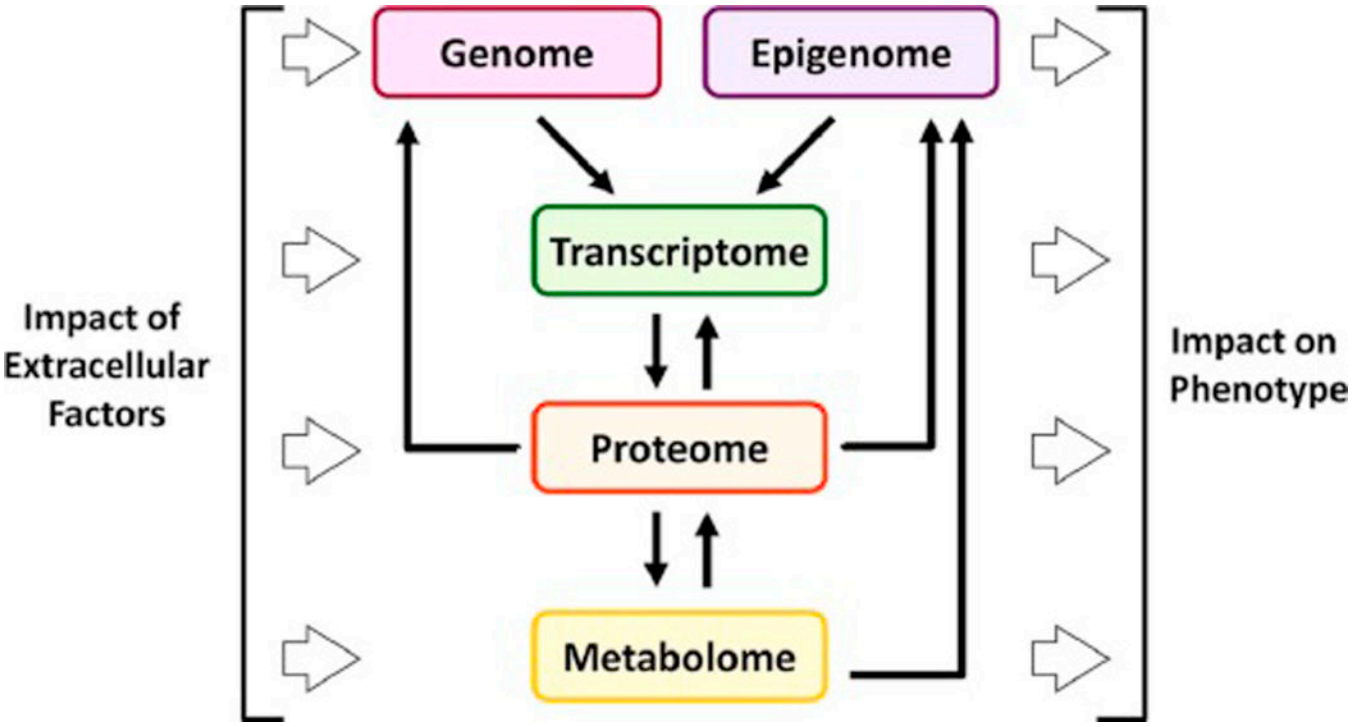


**Figure 1. Schematic representation of how genome, epigenome, transcriptome, proteome and metabolome modulate each other and their impact on the cellular phenotype**

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.

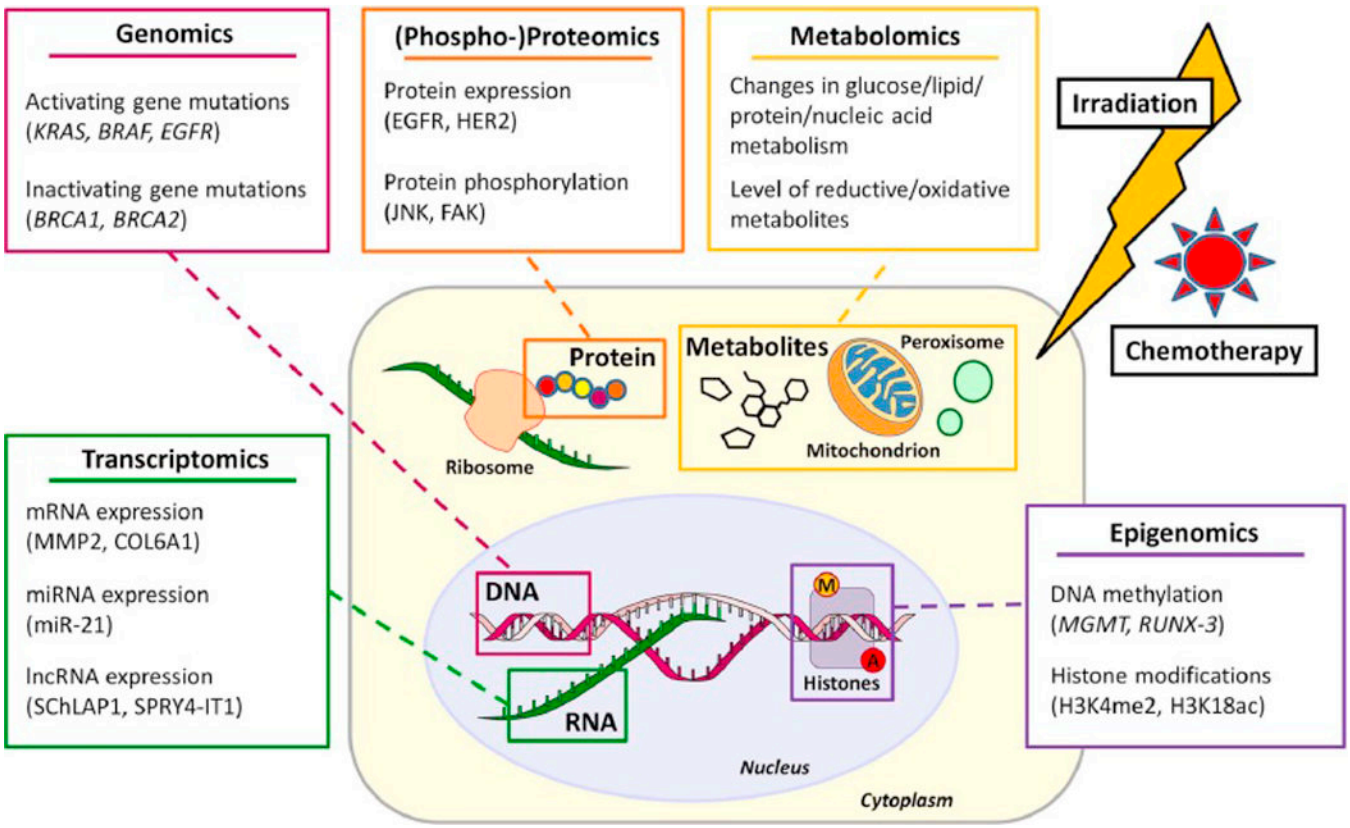


**Figure 2. Omics-based analysis methods and the associated cell processes**

Genomic (activating and inactivating mutations), epigenomic (DNA methylation and histone modifications), transcriptomic (mRNA, miRNA, lncRNA expression), proteomic (protein expression and phosphorylation) and metabolomics (metabolites) analysis is used to clarify resistance mechanisms in tumors. On the one hand, molecular tumor characteristics are used to predict sensitivity to radio-chemotherapy (*MGMT* promotor methylation, miR-21 or HER2 expression); on the other hand, existing molecular differences between tumor and normal cells are exploited to target malignant cells more specifically (*EGFR, BRAF* or *BRCA* mutations).