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Radiogenomics Predicting Tumor Responses to Radiotherapy in Lung Cancer

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The recently developed ability to interrogate genome-wide data arrays has provided invaluable insights into the molecular pathogenesis of lung cancer. These data have also provided information for developing targeted therapy in lung cancer patients based on the identification of cancer-specific vulnerabilities and set the stage for molecular biomarkers that provide information on clinical outcome and response to treatment. In addition, there are now large panels of lung cancer cell lines, both non–small-cell lung cancer and small-cell lung cancer, that have distinct chemotherapy and radiation response phenotypes. We anticipate that the integration of molecular data with therapy response data will allow for the generation of biomarker signatures that predict response to therapy. These signatures will need to be validated in clinical studies, at first retrospective analyses and then prospective clinical trials, to show that the use of these biomarkers can aid in predicting patient outcomes (eg, in the case of radiation therapy for local control and survival). This review highlights recent advances in molecular profiling of tumor responses to radiotherapy and identifies challenges and opportunities in developing molecular biomarker signatures for predicting radiation response for individual patients with lung cancer.
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Lung cancer represents the leading cause of cancer-related deaths in the United States and many Western countries. Although many advances have been made in understanding the molecular pathogenesis and treatment of this disease, the overall 5-year survival rate is 15% to 20%, improving only slightly over the past 20 years.[1] The incidence of lung cancer worldwide continues to rise and is responsible for over 1 million deaths each year.[2] A major contributing factor to the observed high mortality rate is that approximately 50% of lung cancer patients present with metastatic disease. Additionally, 30% to 50% of the patients who present at an earlier stage and are treated initially with surgery or thoracic radiotherapy will die of metastatic recurrence, underscoring the need for more effective systemic therapy.[3] Overall, greater than 50% of all lung cancer patients will require radiation therapy (either for thoracic disease or for extrathoracic metastatic sites).

Approximately 80% all lung cancers are non–small-cell lung cancers (NSCLCs), including adenocarcinoma, squamous-cell carcinoma, and large cell carcinoma. Small-cell lung cancer (SCLC) constitutes approximately 15% to 20% of cases, and the overall incidence of SCLC has been decreasing over the last several years in the United States.[4] SCLC has been recognized to be much more responsive to radiation therapy than NSCLC for nearly 30 years, but the molecular basis for this responsiveness is unknown. In contrast, NSCLC tumors exhibit a wide spectrum of response to radiation therapy. Some patients have a robust response to radiation therapy with long-term local control, whereas others relapse in field even with high-dose treatment. This variance is observed in clinical toxicity as well with some patients experiencing severe, acute, and chronic toxicities from treatment; others experience minimal side effects. Therefore, given the heterogeneity of response to therapy as well as treatment-related toxicities, a method to reliably predict clinical outcome would be of significant benefit in this disease. One approach to solve this problem is to identify biomarkers that can predict response to treatment (the development of "per-

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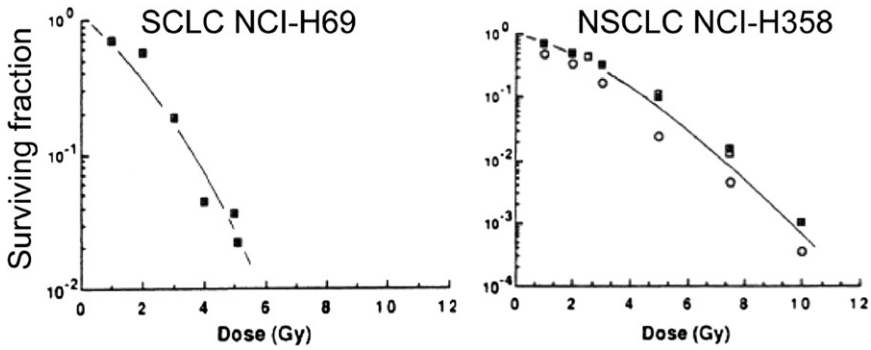

Figure 1 Clonogenic cell survival curves showing SCLC cell line NCI-H69 being more sensitive than NSCLC cell line NCI-H358. (Reprinted with permission.[5])

sonalized medicine.") This article reviews recent advances in identifying lung cancer radiation response phenotypes and potential biomarker signatures predictive of tumor response to radiation alone or integrated with chemotherapy/molecularly targeted therapy.

The Heterogeneity of the Radiation Response Phenotype in Lung Cancer

Lung cancer cell lines vary widely in their radiation response phenotypes. This is evidenced by the large observed variance in the survival of lung cancer cell lines after receiving 2 Gy of ionizing radiation (SF2). In a study of 17 human lung cancer cell lines (14 NSCLC and 3 SCLC), NSCLC cell lines were less sensitive to radiation and had a broader shoulder in survival curves than SCLC cell lines as shown in Figure 1.[5]

Additionally, there was wide heterogeneity in the response to radiation within NSCLC cell lines as shown in Figure 2[5] with SF2 values ranging from 0.17 (sensitive) to 0.93 (resistant).[5] Overall, the SF2 values varied widely among the different histologic subtypes of lung cancer as depicted in Figure 3.[5] Recently, our group has screened a large panel of NSCLC lines for their radiosensitivity and also found that SF2 values varied widely; the most radiosensitive line had an SF2 value of 0.02, whereas the most resistant line had an SF2 of 0.9. Additionally, we found that the radiation response phenotypes of the lung cancer lines were stable over a 20-year period. Although there are clinically approved ways (histology and neuroendocrine biomarkers) to distinguish SCLC from NSCLC (and thus identify the radiosensitive SCLCs), it will be important to develop biomarkers that predict which NSCLC tumors are sensitive and which are resistant to radiation therapy. One approach is to determine if NSCLC lines exhibiting different radiation response phenotypes have distinct biomarker signatures (such as messenger RNA [mRNA] or protein-expression profiles) that are associated with radiation sensitivity that could then be applied to lung tumor samples.

Present Status of Molecularly Targeted Therapy for Lung Cancer

Multiple studies have identified a wide array of genetic and epigenetic alterations in lung cancers, including mutations in DNA sequence, DNA copy number changes, aberrant DNA promoter methylation, changes in mRNA, micro RNA [miRNA] and protein expression, and changes in the tumor microenvironment (such as tumor angiogenesis) revealing many potential determinants and signaling pathways governing lung tumorigenesis and progression.[6] Many of these changes involve oncogenes and tumor suppressor genes.

The proteins encoded by some of these oncogenes are candidates for targeted therapy. The most striking of these are the epidermal growth factor receptor (EGFR) tyrosine kinases inhibitors and the dramatic response associated with their use in lung cancers with somatically acquired EGFR

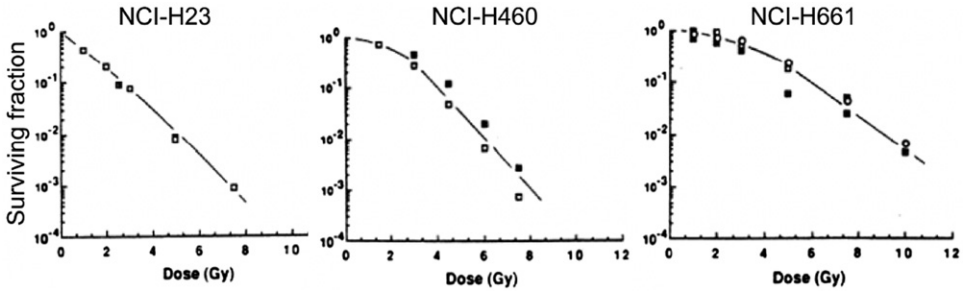

Figure 2 The representative NSCLC cell lines show a significant difference in cell survival as determined by clonogenic cell survival. The most resistant cell line NCI-H661 has broader shoulder than sensitive cell line NCI-H23. (Reprinted with permission.[5])

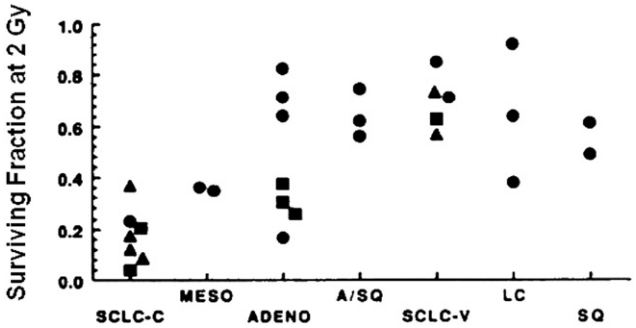

Figure 3 The distribution of SF2 values across different histologic subtypes in 29 human lung cancer cell lines. SCLC-C, classic small cell; MESO, mesothelioma; Adeno, adenocarcinoma; A/SQ, adenosquamous; SCLC-V, variant small cell; LC, large cell anaplastic and SQ, squamous carcinoma. (Reprinted with permission.[5])

mutations (often manifested as either an in-frame deletion in the 19th exon [ΔE746-E750] or an L858R replacement in the 21st exon of EGFR), the use of the monoclonal antibody bevacizumab directed against the vascular endothelial growth factor receptor (VEGFR), and most recently the use of drugs targeted against the anaplastic lymphoma kinase (ALK) in NSCLC patients with ALK activated by a DNA translocation.[4,7,8] Other new targeted therapies are needed because these established therapies are beneficial in less than 30% of all NSCLCs, and in most cases responding patients eventually become resistant (in the case of EGFR by acquiring the secondary EGFR mutation T790M or through mesenchymal epithelial transition factor (MET) amplification providing a bypass of EGFR blockade).[4]

Molecular Changes Associated With the Response to Radiation Therapy of NSCLC

Variations in NSCLC responses to radiation therapy alone or in combination with chemotherapy or molecularly targeted therapy are most likely caused in most cases by the genetic and epigenetic constitution of tumors.[9,10] One study has reported that tumor expression of ERCC1, an important component of the DNA excision repair system, is an important predictor of survival in patients treated with platinum-based therapy or radiotherapy.[11-13]

Likewise, there is preclinical evidence that the expression of mutant EGFR and the activity of EGFR could influence responses to radiation.[14,15] In a panel of 19 NSCLC cell lines (10 cell lines expressing wild-type EGFR and 9 cell lines expressing activating mutations in the tyrosine kinase domain of the EGFR), NSCLC cell lines bearing the tyrosine kinase domain mutant of EGFR were many fold more sensitive to radiation compared with their wild-type EGFR-bearing counterpart as shown in Figure 4.[14,16] Most mutant EGFR NSCLC cell lines and the human bronchial epithelial cells (HBEC) stably expressing mutant forms of EGFR showed delayed radiation-induced DNA repair kinetics as shown in Figure 5. The associated radiosensitivity was attributed to the failure of mutant EGFR to translocate to the nucleus, making it unable to interact with DNA-dependent protein kinase, a key enzyme responsible for the repair of radiation-induced double-strand break.[16]

In contrast to sensitivity to EGFR TKIs, the exogenous expression of mutant EGFR compared with wild-type EGFR dramatically increased lung cancer and normal HBEC radiosensitivity (Fig. 6).[14,16] The EGFR is overexpressed in most NSCLCs and modulate a variety of downstream signaling pathways to communicate from the cell surface to the nucleus, thereby making it an attractive target for the development of EGFR-targeted molecules. Multiple downstream pathways, including Ras-ref-MEK activation of ERK 1/2 to the PI3K-AKT pathway, are thought to play important roles in cancer cell survival.

Furthermore, interference with EGFR tyrosine kinase activity has been shown to inhibit cancer cell growth.[15] One study has reported that treatment with the EGFR inhibitor erlotinib enhanced radiation sensitivity in the presence of wild-type EGFR.[17] In another in vitro study, when radiation therapy was combined with inhibitors of the vascular endothelial growth factor receptor and the EGF receptor, a significant antitumor effect was reported.[18,19]

Studies over the last decade have revealed the molecular details of diverse intracellular pathways that control cancer development. In addition to EGFR and VEGFR, a number of

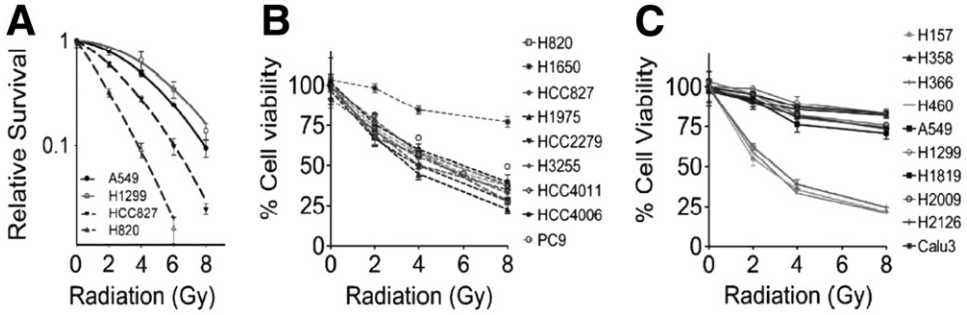

Figure 4 Mutant EGFR-expressing cell lines show enhanced radiosensitivity to radiation. (A) Clonogenic cell survival assay with NSCLC cell lines harboring either wild-type (A549, H1299) or mutant (HCC827, H820) EGFR. Surviving fractions are plotted as a function of dose. (B) Cell viability assay in mutant EGFR-expressing and (C) wild-type EGFR-expressing cell line. Cell viability relative to untreated samples measured on the 7th day after irradiation.[14]

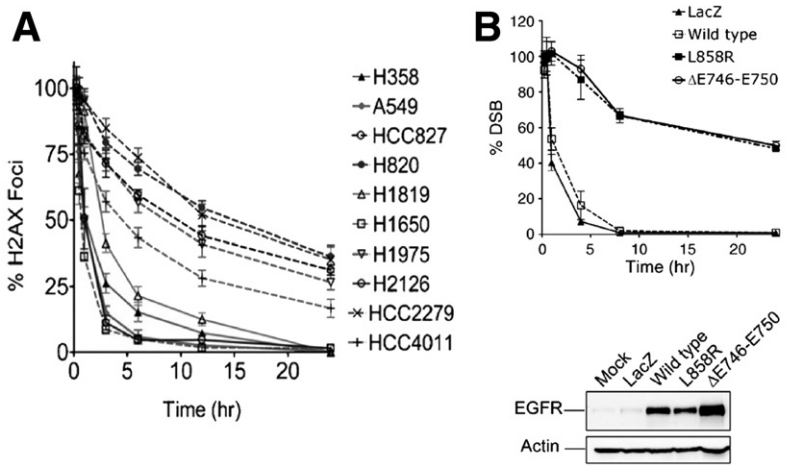

Figure 5 Mutant EGFR-expressing cell lines show slower kinetics of DNA repair compared with wild EGFR-expressing cell lines. A graphic representation of the resolution of phosphorylated γH2AX over time in (A) 4 wild-type (solid lines) and 6 mutant (broken lines) EGFR NSCLC and (B) in HBEC cells stably transfected with a mutant of EGFR.[14,16]

critical components, such as PI3K, have been identified that now serve as targets for the development of novel therapeutics.[4] Similar to EGFR, PI3K is activated in human cancers, and the downstream molecule Akt is constitutively activated in NSCLC and promotes resistance to chemotherapy and radiation. Preclinical studies with the inhibitor of PI3K (LY294002) have shown enhanced sensitivity of NSCLC cells to chemoradiation, and phase I trials are underway.[20] Another major pathway frequently activated in lung cancer is the RAS pathway, and 10% to 15% of the NSCLCs have activating RAS mutations predominantly in KRAS.[4] A variety of agents targeting this pathway are under clinical investigation, and 2 of them (farnesyl transferase inhibitors tipifarnib and lonafarnib) are being tested in combination with cytotoxic chemotherapy.[21,22]

Identification of Biomarkers to Improve the Treatment of NSCLC Patients

Biomarkers can be classified in several ways: (1) diagnostic biomarkers that identify distinct subtypes of cancer, (2) prognostic biomarkers that provide information on patient survival and tumor aggressiveness (which would indicate the need for additional systemic chemotherapy or radiation treatment) and (3) predictive biomarkers that predict the response to particular therapies before the treatment is administered. Biomarkers could include DNA, RNA, protein, miRNA, and metabolic or physiological parameters and could be detected in tumor cells, the tumor microenvironment, blood, or other biospecimens by laboratory or imaging approaches. Of course, the least invasive approaches would

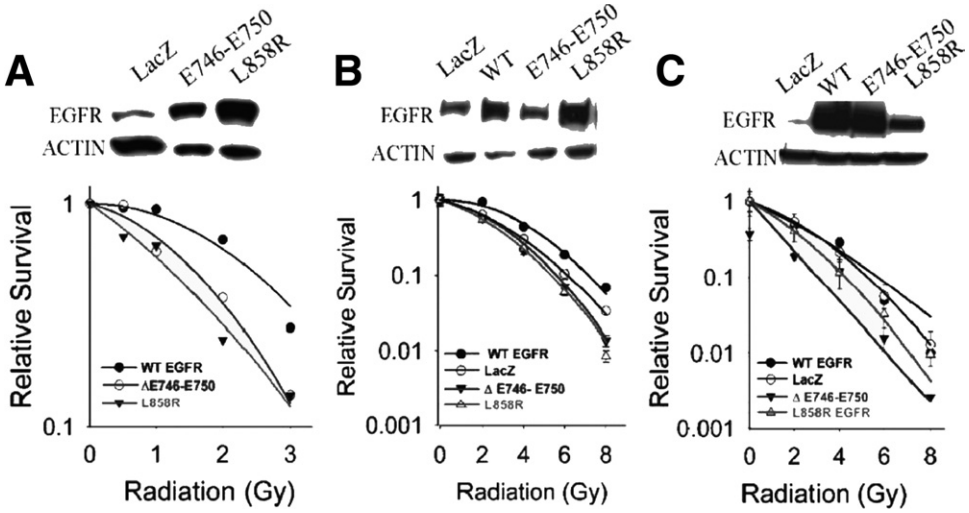

Figure 6 Ectopic expression of L858R or ΔE746-E750 mutant EGFR construct sensitizes cells to irradiation. The effect of stable overexpression of LacZ vector, wild type (WT), L858R, and ΔE746-E750 mutant EGFR on clonogenic cell survival in (A) human bronchial epithelial cells, (B) A549 NSCLC cells, and (C) H1299 NSCLC cells.[14]

be the most feasible to apply in the clinic and may include the analysis of biomarkers in circulating tumor cells.

The identification of early diagnostic and predictive biomarkers in NSCLC has the potential to impact on the overall survival.[23,24] A recent study in tumor xenografts derived from NSCLC patients revealed a high degree of similarity of metastases with the original primary tumor specimen with regard to histology, immunohistochemistry, and overall gene-expression profiling.[25] Although there is a vast amount of literature in existence regarding the development of predictive biomarkers, most of these signatures have not been validated with tumor-derived xenografts. Multiple studies using genome-wide mRNA microarrays to obtain tumor-expression profiles have been implemented (including in lung cancer), and these have identified potential signatures predictive of chemotherapy sensitivity and resistance.[10] Similar studies are needed for the prediction of radiation response.

Biomarkers for Radiation Response

The ability to predict individual tumor radiosensitivity through the development of a predictive assay is central to the development of personalized treatment strategies in radiation oncology. However, there are no validated biomarker signatures addressing radiation response phenotypes in NSCLC. Recently, 3 independent studies of human cancer lines have reported sets of genes involved in radiation response using their models to predict the radiosensitivity based on SF2 values. All groups used cell lines from the NCI-60 panel, which includes a spectrum of tumor types and a relatively small number of lung cancer lines (SCLC and NSCLC).

Torres-Roca et al[26] developed a radiation classifier to predict the radiosensitivity of tumor cell lines based on basal gene-expression profiles. Their classifier correctly predicted the SF2 values in 22 of 35 cell lines. Gene selection identified 3 novel genes, RbAp48, RGS10, and R5PIA, whose expression values correlated with radiation sensitivity. They confirmed the gene expression by quantitative real-time polymerase chain reaction and also biologically validated the results by showing that 3 different tumor cell lines were radiosensitized by overexpression of RbAp48. However, this study had substantial limitations because their panel included only 4 NSCLC cell lines, and they were able to predict correct SF2 values for only 2 of the 4 NSCLC lines evaluated.

Amundson et al[27] reported mRNA signatures obtained by microarray profiling in response to radiation rather than signatures based on basal gene expression. They identified 22 genes differentially expressed in cells with low survival at 2 Gy (SF2 <0.2) and 14 genes associated with cell lines that were more sensitive to 8 Gy (SF8 <0.001) of radiation, including 5 genes that were common to both. They also found 18 genes that were overexpressed in radioresistant cell lines that were presumed to be competent in double-strand break repair (because these cell lines have broader shoulder in survival curves). Interesting and unexplained is that this gene set was not enriched with DNA repair genes except XRCC1. This is contrary to the earlier findings by 2 independent groups (Ross et al[28] and Shankavaram et al[29]) who have reported a consistent relationship between tumor-specific basal gene/protein expression pattern and tissue of origin (organ of origin) in the NCI-60 panel and were able to group specific tumor types according to the organ of origin.[28,29] Amundson et al[27] have reported that radiation-induced gene expression did not substantially differ as a function of tissue of origin.

Amundson et al[27] also identified a set of 25 genes that were preferentially upregulated in tumors with wild-type p53 after radiation, and 15 of these previously had been reported to respond to radiation in a p53-dependent manner. By comparing the SF2 and SF8 (survival fraction at 8 Gy) values as a function of p53 status (wild type vs mutant), the authors did not find any statistically significant difference in tumor cell line clonogenic survival.

As an extension to the Torres-Roca study,[26] Eschrich et al[30] took a systems-biology approach (in which different molecular regulatory networks are organized as complex interacting networks) in understanding radiosensitivity and identifying radiation-specific markers. Their model, based on mRNA expression profiles, identified 10 gene networks or "hubs" (anchored in the different cases by c-Jun, HDAC1, real [p65 subunit of NFKB], PKC-beta, Sumo-1, c-ABL, STAT1, AR, CDK1, and IRF1). Although none of the genes in the 10 hubs were directly related to double-strand repair break, almost all the double-strand break repair genes interacted with the network either directly or indirectly. As validation of one of their larger networks with c-Jun as the hub, they found that when they knocked down c-Jun in 8 different cancer cell lines (lung, colon, and breast cancer), there was an overall trend toward radioresistance, predominantly in lung cancers, but not in the breast or colon cancers, implying that the tissue of origin was important.

They reported a direct correlation between gene expression and radiosensitivity of the lung cancer cell lines and developed a model that identified 4 different clusters of genes that were markers for radiosensitivity. This model also included tumor tissue of origin specificity and KRAS mutation status, which interacted with gene expression to play a critical role in determining cellular radiosensitivity. This model was further used to assess its predictive abilities in the remaining cell lines of the NCI-60 panel and was correct for 5 of the remaining 12 lines. The definition of correct was arbitrary in that the prediction was scored as accurate if it was within 0.10 of the reported SF2 value. These 12-cell lines represented 6 different tumor types from leukemia to central nervous system (CNS). The model did not predict for leukemia or CNS; it was accurate for 1 each of the 2 breast or ovarian cancer cell lines, and was accurate for 2 of the 3 NSCLC lines. Because of the limited numbers of validation cell lines, it is not clear whether the model is poorly predictive or whether tumor-site specificity is driving the results, the lack of accuracy is likely dependent on both. These authors are to be commended for their approach given their decision to predict an SF2 value, and as more cell lines of a specific origin are examined, the question of site specificity will be resolved. It should be noted that none of these differ-

ent signatures have been validated in retrospective or prospective studies of tumors in patients. Furthermore, such discovery-based approaches are potentially associated with biases such as overfitting.[31]

Conclusions

Lung cancers show dramatically different responses to radiation therapy in the clinic, and lung tumor lines also exhibit large quantitative differences in response to radiation in preclinical studies. One striking correlation is the radiation sensitivity of SCLC in patients and in tumor cell lines in vitro.[5,32,33] Other studies in lung cancer and other human cancer cell lines show that there are several biomarkers, including mRNA expression profiles that are associated with radiation sensitivity and resistance. In addition, there are well-known tests of DNA repair and DNA repair–associated factors that can serve as biomarkers in preclinical systems that are potentially predictive of response to radiation. The association of EGFR mutations with radiation sensitivity in preclinical in vitro studies is an important new finding that has potential therapeutic importance. It will be important to see if this radiation response phenotype extends to lung cancers with EGFR mutations in the clinical setting.

Although this review has focused on biomarkers found in tumors, the tumor microenvironment also plays a critical role in determining intrinsic radiosensitivity. Further studies on biomarkers of the relationship between the tumor microenvironment and radiation response are ongoing. Another important component that can potentially influence the tumor radiosensitivity is the cancer stem cell population.[34] In addition, with the availability of high-density single-nucleotide polymorphism arrays, germline genome-wide studies of individual variation in response to radiation, including lung cancer response and radiation toxicity, are in progress. Although the first studies have focused on mRNA profiles, proteomic studies will also be important because mRNA expression is not always correlated with protein expression or posttranslational modification. Of course, it will also be important to develop signatures predicting response to combined modality chemotherapy and radiation therapy. The next big step will be to test the biomarker signatures developed in the tumor cell lines (such as the mRNA signatures) in first retrospective and then prospective clinical studies. For example, it would be of great interest to determine if signatures predicting radiation resistance in NSCLC lines also predict for in-field recurrences of lung cancers (that have received technically appropriate radiation therapy). Likewise assessing biomarker profiles of lung cancers before and after radiation therapy (eg, at the time of local recurrence) to see if the surviving tumor has a signature correlated with radiation resistance would also yield important insights.

Recent developments in high-density genome-wide DNA copy number with high-density single-nucleotide polymorphism arrays, genome-wide methylation status, and genome-wide miRNA expression status are also being studied for association with radiation response. Finally, in the next 3 to 5 years, new DNA sequencing approaches ("NextGen" DNA sequencing) will allow a comprehensive look at the sequence of the total tumor, and the presence of specific mutations may also prove to be biomarkers predictive of sensitivity or resistance to radiation therapy.

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