Biomarkers and Radiotherapy

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Abstract

For a biomarker to be clinically useful there must be adequate preclinical data and have prevalence in the disease of interest. Early research focused on molecules implicated in the cell cycle, DNA repair pathways, and apoptosis as radiation is known to affect such pathways. More recent data has focused on big data, i.e.—omics (genomics, proteomics, etc.) to find a molecular signature that predicts response to radiation as well as identify those who may have increased risk of radiation induced toxicities. While many potential biomarkers in assessing radiation response have been researched this chapter is a start to providing information on biomarkers used in clinical practice.

Keywords

Biomarker · EGFR · HPV · MGMT · PSA · ATM · TGF1-beta · Genomics · Proteomics

1 Introduction

A current goal in medicine is to individualize treatment to eradicate disease while reducing toxicity and improving quality of life. Currently radiation doses are generalized with consensus statements for dose tolerance of normal tissues and local control of tumor based on histology and organ/location. Current radiation dose guidelines are based on laboratory studies of the general radiosensitivity/radioresistance of a

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particular tumor and modeling the tumor control probability (TCP) based on growth characteristics of a tumor. Recently molecular biomarkers allow for personalized treatment approaches and potentially adaptive radiotherapy and or radiation dose escalation/de-intensification. This chapter gives a review of known common biomarkers used in clinical practice today and is sectioned by organ site. The chapter also presents data on the more recent large scale analysis of a patient's molecular profile, i.e.–omics profiling of tumors (genomics SNPs, proteomics, etc.) to predict radiation effects. The goal of biomarker research is to one-day tailor treatment based on an individual's genetic and molecular profile. This book chapter aims to highlight various biomarkers in each cancer type by histology.

2 Head and Neck Cancers

HPV (human papilloma virus)/p16: predictive biomarker. HPV is the most well-known and reproducible predictive marker in head and neck cancer to date (Wierzbicka et al. 2015). It is the most widely discussed marker researched in radiation oncology today. This section focuses on head and neck biomarkers with an emphasis on HPV. Radiation dose de-escalation trials are ongoing in patients with HPV/p16+ head and neck cancer based on the breadth of research on this virus (Wierzbicka et al. 2015).

Traditionally all squamous cell cancers of the head and neck were treated the same with dose guidelines based on tumor size and surgical lymph node drainage patterns. Historically risk factors for head and neck cancer included excessive smoking and alcohol history. More recently there has been an increase in non-smokers with head and neck cancer. The common thread in this subtype of patients has been the prevalence of HPV/p16 in the tumor cells (Chau et al. 2014). HPV is a DNA virus; there are many subtypes. The one subtype of HPV consistently found to correlate with head and neck cancer is HPV/p16. HPV positivity is confirmed by both presence of HPV DNA using PCR and protein overexpression of p16 on immunohistochemical stains (Lee et al. 2015).

In patients with cancer of the oropharynx patients that have HPV p16 positivity tend to present with more locally advanced disease (Ang et al. 2010). In spite of this HPV p16 expression imparts a better response to radiation both standard fractionation and altered fractionation (Lassen et al. 2011, 2014). Lau et al. (2011) demonstrated that p16+ head and neck squamous cell cancer patients had improved overall survival, disease-free survival, and less locoregional recurrence when compared to p16-patients (Lau et al. 2011). Hong et al. (2010) demonstrated that HPV-patients had 13-fold increased risk of locoregional failure and 4-fold increased risk of death as compared to HPV+ patients (Hong et al. 2010). Lassen et al. (2014) showed that the HPV/p16 expression only positively correlates with response in oropharynx patients; p16 expression did not affect outcome of non-oropharynx patients (larynx, hypopharynx) (Lassen et al. 2014). HPV/p16 seems to be more predictive of response to radiation over surgery; Quon et al. (2013) analyzed p16

expression in resectable oropharyngeal carcinoma and found no difference in surgical outcomes of p16+ and p16-patients treated with surgery first (Quon et al. 2013). Future trials underway in head and neck cancer use radiation dose de-escalation in HPV p16 positive oropharynx patients due to this correlation as a predictive biomarker (Ang and Sturgis 2012).

As HPV positivity is established as a predictive biomarker of response to radiation, there are now studies trying to delineate biomarkers that predict for failure in the HPV+ subset (Lee et al. 2015). Inflammatory cells have been evaluated as a marker for predicting treatment failure in HPV+ tonsil cancer and Lee et al. (2015) demonstrated that both overall survival and disease specific survival was affected by high CD68+ and low CD8/CD4 T lymphocyte ratio (Lee et al. 2015). Neither T stage nor N stage were related to outcomes in this HPV+ tonsil cohort (Lee et al. 2015). Extent of inflammation and response to radiation is a common theme and this paper attempts to start the further subtype characterization of HPV+ patients. Other studies have tried to identify a panel of biomarkers that will predict treatment failure, Thibodeau et al. (2015) found that upregulation of LCE3D (late cornified envelope 3D) and KRTDAP (keratinocyte differentiation-associated protein) and down regulation of KRT19 (keratin 19) was observed in posttreatment failures of HPV+ patients (Thibodeau et al. 2015). These biomarkers haven't been extensively studied in radiation and so future studies will be needed to validate these results.

EGFR is another biomarker analyzed in head and neck patients. There have been mixed reports in its ability to predict locoregional control from radiation therapy (Lassen et al. 2013). While the signal for prediction is not as strong as HPV p16 expression, upregulation of EGFR has been shown to correlate with tumor growth and benefits from accelerated radiotherapy (Eriksen et al. 2004). In the DAHANCA 6 and 7 studies, low EGFR expression correlates with high HPV/p16 expression which seems reasonable given that HPV/p16 expression patients respond better to treatment (Lassen et al. 2013). However the signal for EGFR predicting head and neck cancer was not as strong as HPV/p16 expression and so is not routinely recommended for monitoring at this time (Lassen et al. 2013). Recently EGFR was reassessed in HPV+ and HPV-head and neck patients and again demonstrated that EGFR expression did not affect outcomes in HPV+ patients. In HPV-patients, EGFR expression correlated with worse locoregional failure but only in univariate analysis with T and N stage playing more prominent role (Vainshtein et al. 2014).

Similar to EGFR, p53 mutational status has also been analyzed in head and neck cancer patients. Alone p53 mutational status did not affect local control or overall survival but there was a suggestion that p53 mutant head and neck cancer patients may benefit from shortened treatment time similar to EGFR overexpressing patients (Eriksen et al. 2005). Future studies are underway examining EGFR expression, p53 mutational status, HPV/p16 expression, and smoking status to see if there are further subsets of head and neck cancer patients.

Hypoxia molecules have also been studied as predictive biomarkers of radiation resistance mostly because it is known that lack of oxygen makes tumor cells less sensitive to radiation (Overgaard et al. 2005). Osteopontin is one such biomarker

associated with tumor hypoxia. In studies by the DAHANCA group, Overgaard et al. 2005demonstrated head and neck cancer patients with high levels of osteopontin (>167 ug/L) had poorer responses to radiation with higher levels of locoregional failure (Overgaard et al. 2005). In a parallel study at Stanford Petrik et al. (2006) demonstrated that high levels of osteopontin (>450 ng/ml) correlated with higher rates of locoregional failure (3 yr FFR was 72% for patients with osteopontin <450 ng/ml versus 48% for patients with >450 ng/ml (Petrik et al. 2006). Other markers of hypoxia being investigated as markers of radiation resistance include hypoxia inducible factor HIF-2 alpha (HIF-2) and carbonic anhydrase CA9; CA9 is actually one indicator of HIF-1alpha (HIF-1) function. HIF-1 and HIF-2 are thought to be two separate response pathways (Koukourakis et al. 2006). Using data from the CHART trial (continuous hyperfractionated accelerated radiotherapy), Koukourakis et al. (2006) demonstrated that head and neck cancer patients with high levels of HIF-2 and CA9 had worse locoregional control (Koukourakis et al. 2006). These studies haven't led to routine measurement of hypoxic markers or use of hypoxia modifiable treatments such as nimorazole but overcoming hypoxia is still an active area of research in radiation resistance. Future patient samples may well be tested for these hypoxic markers.

From all these various markers only HPV is used routinely in radiation oncology clinical practice in head and neck cancers. Research studies are still underway with these other biomarkers and it is yet to be determined which will be of clinical use in the future.

3 Gynecologic Cancers

Gynecologic and head and neck cancers share many similar biomarkers and thus this next section will highlight some studies of biomarkers in the gynecology literature.

Similar to head and neck cancer, HPV has been implicated in cervical cancer as well and is used as a biomarker. The high-risk HPV 16 and HPV 18 are the most common HPV strains implicated in cervical cancer (Song et al. 2011; Qin et al. 2014). Currently the standard treatment of cervical cancer is concurrent chemotherapy and radiation therapy (Qin et al. 2014). There has been suggestion that for a subset that is radioresistant treatment intensification is needed but finding that subset has remained elusive thus far. Some reports suggest that there is difference response to chemoradiation among the HPV strains (Ferdousi et al. 2010). In one small study of 113 cervical cancer patients, response to radiation was better in HPV-58 and HPV-31 versus HPV 16 and HPV-33 (Ferdousi et al. 2010). There have been reports that persistent HPV after definitive radiation for cervical cancer may predict worse local control of disease (Song et al. 2011). Song et al. (2011) showed that persistent HPV DNA 24 months after radiation predicted risk of local recurrence and HPV persistence at just 3 months alone was the earliest predictor of local recurrence (Song et al. 2011). Testing for HPV is routinely done in clinical

practice and this data suggests that all patients treated for cervical cancer with radiation should have HPV testing after radiation is complete as well. Patients with persistent HPV may need treatment intensification either in form of altered radiation treatment regimens, or adjuvant chemotherapy. This data still needs to be validated in multi-institutional trials before becoming routine use in clinical practice.

EGFR has also been explored as a biomarker in cervical cancer in the same manner as it has been studied in head and neck cancer (Qin et al. 2014). Overexpression of EGFR has been shown to lead to more failures after definitive radiation suggesting it is a predictive biomarker of radiation resistance (Pérez-Regadera et al. 2011). Perez-Regadera et al. (2011) examined 112 cervical cancer biopsies and found that patients with high overexpression of EGFR on biopsy had more pelvic relapses and decreased disease free survival with hazard ratio of 2.31 (Pérez-Regadera et al. 2011). Cerciello et al. (2007) demonstrated that changing EGFR levels during radiotherapy administration did not have any correlation with response though they did not mention quantification of initial expression of EGFR (Cerciello et al. 2007). Thus, EGFR may be a biomarker only of inherent radiation resistance and from these studies it suggests that initial EGFR expression of tumor may be more significant in predicting radiation resistance. EGFR testing in cervical cancer is not routinely done currently but may be considered in future trials arguing for more intensive treatment of radioresistant tumors.

Other biomarkers being tested include the bcl2 apoptotic family members such as BAX, prostaglandin pathway molecules such as COX, and hypoxic markers such as HIF1alpha (Qin et al. 2014). Currently only HPV is routinely screened prior to radiation therapy in cervical cancer but these other biomarkers may become significant as we try to individualize treatment or argue for treatment intensification in radiation resistant cervical cancer subtypes.

4 CNS

Glioblastomas are the most aggressive brain tumor. Historically treatment was surgery and whole brain radiation. With the advent of chemotherapy and better imaging with MRI brain, radiation in glioblastoma is directed at the tumor. A landmark trial demonstrated the benefit of temozolomide with limited field radiation in improving overall survival (Stupp et al. 2009).

Current evidence has demonstrated that not all glioblastomas are the same and survival varies widely. New reports suggest specific molecular subtypes have better survival. In the landmark Stupp trial, subset analysis of this trial demonstrated that patients with MGMT methylation have double the survival at 5 years (Stupp et al. 2009). The MGMT (O6-methylguanine-DNA-methyltransferase) gene encodes a DNA repair protein. Methylation of the MGMT promoter silences the gene and prevents DNA repair namely of damage caused by alkylating agents. Thus, this gene is important for regulating the DNA integrity of the cell. MGMT methylation has been shown to sensitize glioblastoma cells to temozolomide, an alkylating agent

and is thus a predictive biomarker of the chemotherapy response. Rivera et al. (2010) asked the question if MGMT methylation also sensitizes cells to radiation as radiation also works primarily through DNA damage (Rivera et al. 2010). 225 patients were analyzed in their study of glioblastoma patients who received radiation alone after maximal safe surgical resection (i.e. no chemotherapy such as temozolomide) (Rivera et al. 2010). They demonstrated that patients with MGMT methylated had better response to radiation and that unmethylated tumors were twice as likely to progress during radiation treatment. On multivariate analysis, methylation was independent of age, KPS, and extent of surgical resection (Rivera et al. 2010).

MGMT is now used routinely in clinical practice as both a predictive and prognostic biomarker for chemotherapy. New strategies in glioblastoma treatment involve using MGMT methylation to alter upfront therapy, i.e. adding other targeted agents such as everolimus, etc. that work through pathways different than temozolomide to sensitize glioblastoma cells to radiation. The goal is more tailored treatment for glioblastoma subtypes in an effort to more efficiently eradicate tumor cells. Recently a Phase III randomized control study (GLARIUS trial) was published showing that altering chemotherapy in MGMT methylated patients could improve progression free survival (Herrlinger et al. 2016). In this study, non-MGMT methylated (i.e. predicted temozolomide and radiation resistant) patients with newly diagnosed glioblastoma were randomized to standard of care temozolomide + radiation versus bevacizumab+irinotecan+radiation (Herrlinger et al. 2016). However, the study failed to show improved overall survival as the original Stupp trial so temozolomide and radiation is still the standard for glioblastoma patients (Stupp et al. 2009; Herrlinger et al. 2016). Future studies may target radiation dose escalation or altered radiation fractionation schedules such as hypofractionation or stereotactic body radiation doses.

Recent studies by Ahmed et al. (2015) have looked into generating a radiosensitivity index (RSI) for different cancer subtypes including glioblastoma (Ahmed et al. 2015). The RSI described previously by the same group uses gene expression patterns, tissue histology, and ras and p53 status when cells are treated with radiation (Ahmed et al. 2015; Eschrich et al. 2009). The RSI index directly correlates with tumor radioresistance (high RSI = radioresistance) (Ahmed et al. 2015; Eschrich et al. 2009). Ahmed et al. (2015) used the TCGA (the cancer genome atlas) which has large population data based on histology and centralized at the NIH to see if RSI could predict radiation response (Ahmed et al. 2015). RSI was a predictor of overall survival for the glioblastoma cohort (Ahmed et al. 2015). For radiosensitivity predictions RSI correlated with response in patients with high MGMT expression (Ahmed et al. 2015). MGMT is already known to predict radiation response so it will be interesting to see if the further information from RSI can help delineate earlier which patients will need treatment intensification.

There are now known to be three subclasses of glioma: proneural, proliferative, and mesenchymal. By using these subtypes, known biomarkers such as MGMT and new genomic profiles such as RSI we can begin to tailor treatment for glioblastoma.

5 GU

Prostate cancer is the second leading cause of cancer deaths. Although prognostic factors such as clinical T stage, Gleason score and pretreatment PSA aid in prognosis of prostate cancer there are still many outliers. Early risk prostate cancer can fail localized therapy such as radiation earlier than planned and high risk prostate cancer can remain seemingly indolent for years. Other prognostic and predictors of radiation response in prostate radiation therapy are needed.

PSA is the single most used test in prostate cancer. It is used to screen men though its use as a screening tool has come into question given its high sensitivity and over-diagnosis of slow growing prostate cancers. Elevated PSA (generally >4) prompts urological consult and prostate biopsy. PSA response after definitive treatment (i.e. either surgery or radiation) is the most sensitive test and predicts progression free survival long before patient develops any recurrent tumor or metastases.

Kabarriti et al. (2014) and colleagues wanted to test if PSA can be used during radiation treatment to predict response. Such a marker would give patients confidence in radiation alone as salvage treatment and less worry about earlier need for additional salvage treatment such as hormonal treatment (i.e. lupron) or chemotherapy (i.e. docetaxel). Kabarriti et al. (2014) demonstrated that PSA response during radiation is a predictive biomarker of outcome of salvage prostatectomy patients (Kabarriti et al. 2014). 5 year biochemical control rate for PSA responders was 81% compared to 37% for non-responders (Kabarriti et al. 2014). This suggests that PSA should be used during radiation treatment to give an earlier predictor of patient outcome. If PSA is not responding adequately during radiation dose escalation could be considered or earlier use of additional chemotherapy may be warranted.

Another more recent biomarker is the genome prostate cancer classifier (GC) (Den et al. 2014). The GC score developed by Den et al. (2014) utilizes-omics data, specifically gene expression patterns with microarrays (Den et al. 2014). This GC score helps to predict which men would benefit from earlier adjuvant radiation versus delayed salvage radiation when frequently PSA is higher and radiation may be of less benefit (Den et al. 2014). The A high GC score predicted increased biochemical failure and metastases thus suggesting these men need more aggressive systemic therapy (i.e. long term hormones) (Den et al. 2014).

In lines with the GC score, research has focused on pretreatment molecular characteristics of the prostate cancer to determine if radiation as localized treatment should even be attempted or if patient should go to surgery. P53 accumulation and high expression in prostate cancer cells seems to predict radiation treatment failure in many prostate studies reported by independent research groups (Ritter et al. 2002; Scherr et al. 1999). Abnormal p53 expression was then analyzed in a multi-institutional RTOG trial, RTOG 8610 (Grignon et al. 1997). In this trial, all patients received radiation as the local treatment for prostate cancer and the phase III randomization was for ± addition of androgen deprivation (i.e. zoladex and

flutamide) (Grignon et al. 1997). Abnormal p53 expression led to decreased time to development of distant metastases and increased incidence of distant metastases though these results must be taken with caution as they only demonstrated that p53 expression only affected response in patients who received both androgen deprivation and radiation and this was in an era without prostate radiation dose escalation which is standard today (Grignon et al. 1997).

Although in general prostate cancer is thought to be slow growing and with low proliferation index, there is a rare subtype of prostate cancer with a high proliferation index as measured by Ki-67 (Pollack et al. 2004). Pollack et al. (2004) analyzed Ki-67 expression in prostate cancer biopsies of men enrolled in a multi-institutional phase III randomized trial RTOG 92-02 (Pollack et al. 2004). In this trial, prostate cancer patients with locally advanced prostate cancer (intermediate and high risk) where randomized to long-term or short-term androgen deprivation concurrent with radiation therapy (Pollack et al. 2004). Pollack et al. (2004) demonstrated that high Ki-67 (cutpoint 7.1%) in prostate predicts poor response to treatment and these patients had higher biochemical failure, distant metastases and cause-specific death (Pollack et al. 2004). Future studies would need to aim at better initial treatment for this aggressive subtype of prostate cancer maybe with upfront plan for trimodality treatment versus trying one localized treatment and watching/waiting.

Most of these studies are looking at biomarkers that can predict response to radiation. There have been recent efforts in prostate cancer to also see if biomarkers can predict radiation toxicities. Genome-wide association studies have been used to identify SNPs (single nucleotide polymorphisms) associated with a common radiation toxicity from prostate cancer, erectile dysfunction (ED) (Kerns et al. 2010). This large scare-omics project genotyped 909,000 SNPs of African-American men treated with external beam radiation for prostate cancer (Kerns et al. 2010). The cohort filled out the Sexual Health Inventory for Men (SHIM) questionnaire. SHIM score of ≤ 7 was used to identify men with ED and to see which SNPs correlated. Kerns et al. (2010) identified SNP rs2268363 which is in the follicle-stimulating hormone receptor (FSHR) gene as correlating with ED (Kerns et al. 2010).

Overall PSA is still the main biomarker for prostate radiation. GC score and SNP profiling which uses large scale genomic data to predict individual patient responses to treatment may become more mainstream in the future for prostate cancer versus trying to identify one or two biomarkers.

In terms of other GU cancers there is emerging data on biomarkers predicting response to radiation. Koukourakis et al. (2016) examined tissue from 66 bladder cancer patients treated with hypofractionated accelerated radiation (Koukourakis et al. 2016). They observed that high expression of two biomarkers they analyzed, HIF1alpha and LDH5 correlated with poor response to radiation (Koukourakis et al. 2016). LDH5 (lactate dehydrogenase 5) is part of the anaerobic glycolysis pathway and does not require oxygen to function. The other, HIF1alpha, is part of the hypoxia signaling pathway discussed above suggesting still a common thread to predicting response to radiation. Future studies are needed to validate these findings in a larger cohort of bladder cancer patients.

6 Breast

It is now known that there are many subtypes of breast cancer (luminal A, luminal B, basal type, Her2Neu subtype) (Langlands et al. 2013). Each subtype of breast cancer has different overall survival, risk of metastases, response to chemotherapy and radiation. Recent data discussed below suggests that the specific subtype of breast cancer can predict radiation response (Langlands et al. 2013).

Although it is known that radiation tends to work better in cells that are undergoing rapid cell division it has also been suggested that radiation is more effective for luminal A and estrogen depending breast cancers in reducing risk of relapse (Wang et al. 2011; Kyndi et al. 2008). One theory is that estrogen hastens the cell cycle in the G1 to S transition and that could make cells with more error-prone DNA. Radiation thus would more effectively kill these cells with impaired DNA. For basal subtype (triple negative) breast cancers, many of which possess DNA damage repair deficiencies by virtue of BRCA mutations, there is still high risk of local recurrence even with radiation. There is speculation that there may be aberrant upregulation of alternate DNA damage repair pathways intrinsic to these BRCA mutant breast cancers that are able to overcome radiation induced DNA damage (Langlands et al. 2013).

Triple negative breast cancer is not as responsive to treatment as hormone positive breast cancer. Biomarkers unique to this breast cancer subtype may impart more information as to the mechanism of radioresistance (Speers et al. 2016). From studies of triple negative breast cancer cell lines a new potential radioresistance marker MELK (maternal embryonic leucine zipper kinase) has shown promise (Speers et al. 2016). MELK is overexpressed in triple negative breast cancer cell lines and when inhibited cells become more radiation sensitive (Speers et al. 2016). These laboratory bench studies could be translated into clinical trials assessing MELK as a biomarker. Langlands et al. (2014) also discussed work documenting that high expression of a proteasome subtype (PSMD9) was associated with increased local recurrence in patients that received adjuvant radiation versus patients that did not receive radiation suggesting some association with radiation treatment (Langlands et al. 2014). Another study demonstrated that high levels of peroxiredoxin-I was associated with high local recurrence after radiation (Woolston et al. 2011). Peroxiredoxin-I is in the pathway that regulates oxidative stress and thus may be another pathway that protects cells from radiation damage (Woolston et al. 2011). These would all need to be validated before use in routine clinical practice.

Known radioresponsive gene pathways have also been investigated in breast cancer. A recent paper highlights this by investigating 22 genetic variants in 18 radioresponsive genes and their association with breast cancer radiation reactions, specifically skin damage severity ($\geq$ grade 2 toxicity by RTOG criteria) (Mumbrekar et al. 2016). The authors found that a SNP in CD44 rs8193 with significantly associated with radiation induced skin reactions (Mumbrekar et al. 2016). CD44 positivity has been implicated before as a potential marker of breast cancer stem

cells; there could possibly be a link with CD44 and ability of breast cancer cells to regenerate skin though more work is needed to justify this conclusion (Shao et al. 2016). Further work in specific radioresponsive genes is ongoing.

There has been large scale—omics studies in breast cancer patients to predict toxicities. Ho et al. (2007) initially ran an analysis of DNA sequence alterations in ATM (ataxia-telangiectasia) in breast cancer patients with grade 2 breast fibrosis toxicities from radiation (Ho et al. 2007). ATM is important in regulating the cell cycle and has been implicated in radiosensitivity for many years (Ho et al. 2007). They discovered that a SNP variant of ATM (5557 G → A polymorphism) was associated with increased risk of breast fibrosis (≥ grade 2 late radiation response). This SNP results in a non-conservative amino acid substitution from an aspartic acid to an asparagine in exon 37 in the ATM protein (Andreassen et al. 2016). While the study was small with 131 patients, it suggests that genome wide assays may help pinpoint predictive radiation markers in breast cancer (Ho et al. 2007). There have been many small studies exploring this SNP but no definitive data. A more recent study again looked at ATM SNPs and toxicity in a large cohort of 5456 breast and prostate cancer patients; 2759 patients received radiation for breast cancer and 2697 patients received radiation for prostate cancer (Andreassen et al. 2016). The same SNP discussed by Ho et al. (2007) ATM SNP rs1801516 was associated with radiation toxicity; in this study the association was stronger with acute toxicities (odds ratio 1.5) versus late (odds ratio 1.2) (Andreassen et al. 2016). This study was conducted by the International Radiogenomics Consortium (RgC) and more studies are expected on other cancers from this large multi-institutional collaboration (Andreassen et al. 2016). The authors conclude that such large scale studies are needed to detect weak signals in heterogeneous cohorts. These studies will help pinpoint relevant SNPs that could then be tested in the clinical setting.

7 Thoracic

The standard of care for locally advanced lung cancer is radiation or combination of chemotherapy and radiation. It is already known that small cell lung cancer and squamous cell non-small cell lung cancer respond more to chemoradiation versus non-small cell lung cancer with adenocarcinoma histology. Beyond this it is now known through analysis of the TCGA that lung cancer tends to carry more genetic mutations than was previously appreciated with just histology delineation and that mutations in lung cancer are unstable and can change over course of treatment or after 1st line treatment complete (Kan et al. 2010; Pikor et al. 2013). Predicting treatment response with this now appreciated wide array of non small cell lung cancer subtypes will be helpful to individualize treatment strategies.

Due to this plethora of mutations many studies to predict treatment response now use—omics data to find unique molecular signatures. One such study by Walker et al. (2015) used proteomics and assessed locally advanced non small cell lung

cancer patients that had survived <14 months versus >18 months (Walker et al. 2015). Of 650 proteins analyzed they found that two proteins, CRP (C-reactive protein) and LRG1 (leucine-rich alpha-2-glycoprotein), were highly significant for extended survival when tested for high expression just one week post completion of standard radiation treatment. Less is known about LRG1 but there have been studies suggesting role in angiogenesis and can modulate TGF-beta, a known marker of inflammation (Walker et al. 2015). CRP is an acute phase reactant along the same pathway as IL-6 and correlates with previous studies implicating IL-6 in predicting survival to radiation (Walker et al. 2015). The hypothesis is that over-expression of acute phase reactants is detrimental to radiation response probably through excess toxicity via inflammation. This is an interesting study because it is trying to find early predictive markers to help guide patient treatment decisions. Generally, our first assessment of lung cancer patient's response to radiation is 3 months after completion of treatment but this early biomarker may help in deciding if treatment intensification is warranted (Bradley et al. 2015). Or as these markers may be involved in acute phase inflammation if early treatment to prevent inflammation such as pneumonitis should be initiated earlier rather than waiting to see if patient develops clinical symptoms.

Another pathway recently implicated in response to radiation is immune system activation especially with the advent of immunomodulator treatments concurrent with radiation for the treatment of advanced non small cell lung cancer. In a small study by Deng et al. (2016), the authors reported that high GM-CSF (granulocyte-macrophage colony stimulating factor) levels during radiation correlated with better overall survival and progression free survival (Deng et al. 2016). They also described a new test called the "integrated factor" that takes into account the degree of upregulation of GM-CSF as well as pre radiation levels of another immune pathway molecule IFN-gamma (interferon-gamma) and found this also correlated with prediction of better overall survival and progression free survival (Deng et al. 2016).

The main concerns of radiation to the lung are toxicities to lung itself or other structures of the mediastinum (esophagus, great vessels, heart). Acute pneumonitis and subsequent lung fibrosis are two main concerns of lung damage. Early studies assessed specific biomarkers known to be associated with inflammation. Zhao et al. analyzed the predictive role of TGF-beta1 a known inflammation maker with stage I-III lung cancer patients (Zhao et al. 2008). High levels of plasma TGF-beta1 4 weeks during radiation treatment was significantly predictive of $\geq$ grade 2 pneumonitis or fibrosis (Zhao et al. 2008). Kim et al. (2009) also observed that TGF-beta1 was significantly higher 4 weeks after radiation in patients who developed symptomatic radiation pneumonitis (Kim et al. 2009). Another more recent study looking at esophageal patients treated with radiation who would also have significant dose to lung also showed that plasma TGF-beta1 levels were elevated in patients who developed radiation pneumonitis (Li et al. 2015). Although these three studies suggested TGF1-beta as a biomarker, another small study could not find a correlation between either TGF1-beta or IL-6 and radiation pneumonitis (Rübe et al. 2008). In this negative study by Rube, the authors said that baseline

TGF1-beta and IL-6 levels were already elevated and there was no significant increase after radiation was complete (Rübe et al. 2008). As mentioned the molecular signature of lung cancer is now known to be highly variable and this can confound the data from these small studies. All these studies had small samples sizes and thus before we could use TGF-beta1 in the clinic, expression of this marker would need to be assessed in large scale lung cancer RTOG studies such as the recently completed RTOG 0617 (Bradley et al. 2015).

The other main toxicity in lung cancer is esophagitis (Bradley et al. 2015). The same biomarker TGF1-beta1 was assessed in lung cancer patients. This time Guerra et al. (2012) assessed SNPs in TGF-beta1 in 97 NSCLC patients (Guerra et al. 2012). They found that the SNP rs1800469: C-509T was significantly associated with higher risk of $\geq$ grade 3 radiation induced esophageal toxicity (Guerra et al. 2012).

Novel biomarker studies in lung cancer involve assessing micro RNAs (miRNA) (Dinh et al. 2016). miRNAs are small-non coding RNAs that are now known to function in silencing of RNA and regulation of gene expression. A small study of five patients with stage IIIA NSCLC at 5 different dose points during radiation was analyzed. miR29a-3p and miR-150-5p were shown to decrease as the radiation dose increased during course of treatment (Dinh et al. 2016). miR-150 has been shown to decrease in plasma in animals exposed to radiation (Dinh et al. 2016). miR-29a has already been associated with fibrosis in heart, lung and kidneys and so the authors hypothesize that extreme outliers of levels of these specific miRNAs may help predict toxicity to radiation (Dinh et al. 2016). Radiation dose could then be adapted based on the individual. This theory will be exciting to test in future studies of miRNAs because it will allow radiation oncologists to sculpt dose to tumor with confidence.

8 Intrinsic Radiosensitivity of Tissue/Organs

To predict response to radiation and to prevent toxicities we can look for predictive biomarkers in multiple ways either with a targeted approach of molecules implicated in affecting radiation or with a large scale, large data "-omics" type approach. The early studies focused on intrinsic cellular radiosensitivity (Williams et al. 2007). These studies formed the basis of calling some histologies radiation sensitive and some histologies radiation resistant. These studies looked at known oncogenes and tumor suppressor genes such as ATM and p53 and categorized cancer cell lines based on these genetic characteristics (Williams et al. 2007). ATM (ataxia telangiectasia mutated) was already implication in radiation sensitivity as individuals with AT (ataxia telangiectasia) are highly sensitive to radiation damage (Tribius et al. 2001). For radiation sensitive cancers there was less push for dose escalation whereas for radiation resistant there was more push for dose escalation and newer radiation techniques such as stereotactic body radiation (SBRT).

Another focus more recently has been on aging and the fact that intrinsic cellular radiosensitivity may actually change over time due to environmental exposures and oxidative damage (Mishra et al. 2012). Pathways studied include IL-6, CRP, TGF-beta1, advanced glycation end products (AGE), markers of inflammation. Telomere length is also being studied as we know that shorter telomere lengths over time lead to chromosomal instability and thus in theory more susceptibility to radiation damage (Mishra et al. 2012). Some of these pathways have been researched to assess a patient's "biological age" versus "chronological age" to aid in treatment management decisions. Historically biological age was a variable used to assess if radiation is warranted in certain cancers such as breast cancer. Chronological age may be more accurate in determining who best benefits from radiation.

9 Assessing Biomarkers in Clinic

Testing for biomarkers includes methods employed in the pathology laboratory including but not limited to: immunohistochemistry, mass spectroscopy, mutational analysis and gene expression analysis. The RTOG has a central storage for biospecimens and is a useful tool for testing biomarkers researched in basic science laboratories. Future assessments frequently use patient's serum which is also being banked in ongoing RTOG/NRG studies and validation of biomarkers with these large multi-institutional patient sample banks is necessary before a biomarker can be considered for routine clinical testing. As shown in sections in this chapter patient serum is being used to detect genomic and or molecular signatures of response to radiation through large scale—omics studies (genomics, proteomics).

10 Conclusion

While this chapter is by no means exhaustive of all biomarkers, this goal of the chapter is to highlight some common biomarkers and or genre of biomarkers researched to date to help guide current radiation practices. The goal of predictive radiation biomarkers is to help with individualizing radiation therapy. It will be exciting to see in coming years how big data, -omics, will help advance the field of radiation biomarkers.

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