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Chapter 2

Biomarkers in Lung Cancer: Integration with Radiogenomics Data

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1. Introduction

Lung cancer remains as one of the most aggressive cancer types with nearly 1.6 million new cases worldwide each year. There are an estimated 222,520 new cases and 157,300 deaths from lung cancer in the United States in 2010 [1]. Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer, comprising three major histological subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Chronic exposure to carcinogens drives genetic and epigenetic damage that can result in lung epithelial cells progressively acquiring growth and/or survival advantages, giving as a result the generation of tumor cells. Studies have shown that some specific molecules contribute to sporadic tumors of lung cancer; even now, they are useful as predictive biomarkers. Mutations in at least one of the established lung cancer driver genes including *egfr, kras*, *braf, her2, akt1, nras, pik3ca, mek1, eml4-alk* and *met* amplification are found in approximately 60% of tumor specimens, and greater than 90% were "exclusive": only one mutation was found in a particular tumor [2]. Epidermal growth factor receptor (EGFR) exhibits overexpression or aberrant activation by mutations in 50 to 90% of NSCLC. Much effort has been focused on the development of targeted molecular inhibitors for this molecule, but it has become clear that molecular-targeted cancer therapies can only reach their full potential through appropriate patient selection. Conventional therapies as chemo- and radiotherapy continue being the first option of treatment for lung cancer patients, even their mutation status of NSCLC driver genes. Radiotherapy, alone or in combination with surgery, chemotherapy or biological therapies, play a critical role in the management of lung cancer. Currently, there are several clinical studies in radiation response of NSCLC tumors, which exhibit a wide spectrum of response to this modality treatment. Thus, a successful radiation sensitivity assay to calculate individual tumor radioresponse is central for the development of personalized strategies in radio-

oncology. Some research groups have done effort in radiogenomics and proteomics in lung cancer with the purpose of finding specific molecules to predict resistance or sensibility to radiotherapy. NSCLC tumors with mutations in well-known molecular markers as EGFR and KRAS represent two molecularly distinct tumor entities, with different clinical behaviors. In this chapter we focus on the biomarkers used as biological therapy targets in lung cancer and their impact on resistance to therapeutic interventions. Moreover, we highlight genomic and proteomic data in radiation response to lung cancer.

2. Lung cancer

Lung cancer remains as one of the most aggressive cancer types with nearly 1.6 million new cases worldwide each year. In 2010, in the United States were estimated 222,520 new cases and 157,300 deaths from lung cancer [1]. Non-small cell lung cancer (NSCLC) subtype represents 85% of all cases of lung cancer, while small cell lung cancer (SCLC) subtype comprises 15%. Histologically, NSCLC is classified as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. This classification has important implications for the clinical management and prognosis of the disease [3]. Yet early detection methods are not extensively used in the wider population, malignancy is most commonly diagnosed at a late stage resulting in poor patient survival. Overall 5-year survival rates for lung cancer vary globally but are consistently low (7.5-16%) [1]. Approximately 40% of patients with advanced unresectable disease at the time of diagnosis have a poor prognosis. At present, no single chemo-radiation therapy regimen can be considered standard; despite the treatment choice for unresectable stage III NSCLC, a platinum-based chemotherapy regimen and thoracic radiation are concurrently administered. Chemotherapy concurrently with chest radiation therapy significantly improves the survival of patients with unresectable stage IIIA and IIIB disease. Decades of research have increased understanding the lung cancer as a multistep process involving genetic and epigenetic alterations, through which, resulting DNA damage transforms normal epithelial cells that progressively acquire growth and/or survival advantages until cancer arises [2,4-7]. Malignant transformation of lung epithelial cells is characterized by genetic instability, which can exist at the chromosomal level (with large-scale loss or gain of genomic material, translocations, and microsatellite instability) or at the nucleotide level (with single or several nucleotide base changes). Moreover, lung cancer is also related to genomic and epigenomic changes at the transcriptome (with altered gene and microRNA expression) and proteome [8-11] level. As many kinds of tumors, molecular abnormalities in lung cancer cells are typically targeted to proto-oncogenes, tumor suppressor genes, DNA repair genes, and other genes that can promote outgrowth and immortality of affected cells [12,13]. It is accepted that the successful discovery, validation and implementation of specific molecular markers for early diagnosis, clinical surveillance and determination of tumor response to therapeutic intervention could improve survival rates for patients, but only few biomarkers turned out to be useful in the clinic. *egfr* and *kras* gene mutations are prognosis markers in NSCLC [2,12,14]. Because of the importance of EGFR as a prognostic factor in NSCLC, mutated EGFR has been the target for development of biological therapies; at present, these therapies are being used in treatment of a certain group of patients [15]. In this context, current research focuses on identifying other

potential molecular targets for the development of new agents and the assessment of better combinations of established therapies. Intensive research has originated numerous potential lung carcinoma molecular biomarkers related to therapy response in order to establish an appropriate molecular selection of patients, with focus on personalized medicine.

3. Genome biomarkers: The opening to personalized medicine in lung cancer

Nowadays, molecular and genetic studies have shown that some specific molecules contribute to sporadic tumors of lung cancer; they are useful as therapeutic targets and predictive biomarkers [16]. Recently, the National Cancer Institute's lung cancer mutation consortium (NCI´s LCMC) performed such a study on more than 800 lung adenocarcinoma tumor specimens, examining mutations in established lung cancer driver genes (*egfr, kras, braf, her2, akt1, pik3ca, mek1, eml4-alk, met* amplification) [2]. Mutations in at least one of these genes were found in approximately 60% of tumor specimens, and greater than 90% were "exclusive", namely, only one mutation was found in a particular tumor. EGFR regulates important tumorigenic processes, including proliferation, apoptosis, angiogenesis, and invasion. EGFR, along with its ligands, is frequently overexpressed during the development and progression of NSCLC. *egfr* gene are amplified and over-expressed in 6% of NSCLC. However, activating mutations in exons 18 to 21 comprised in the kinase domain of EGFR (Figure 1) occur early in the development of adenocarcinomas with clinic characteristics like never-smoking, female sex and Asian ethnicity [7,15].

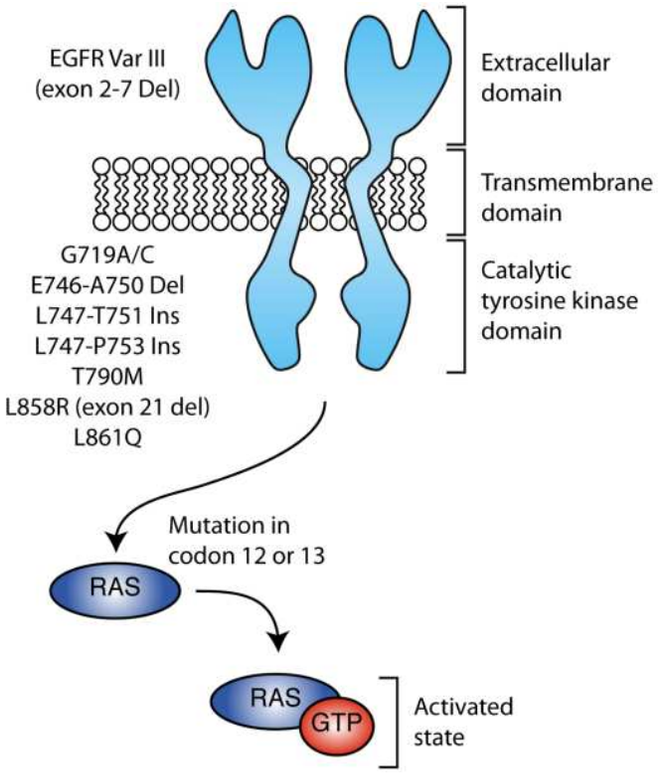

Figure 1. EGFR and KRAS mutations in NSCLC. Mutations in extracellular domain of EGFR have been implicated in resistance to treatment with mAb against EGFR. Mutations in TK domain are most

common in NSCLC, including L858R and E746-A750 deletion in exon 19. These mutations are target for small molecules inhibitors of tyrosine kinases domain (TKI). T790M is a mutation related to resistance to TKI treatment. Mutations in codon 12 or 13 of *kras* gene can lead to constantly union of GTP to KRAS protein, this represent the activate state of KRAS. GTP/KRAS induces activation of signaling depending to KRAS, permitting uncontrolled cell proliferation.

Mutated EGFR are present in 10-15% of NSCLC tumors [2,17]. Mutant EGFRs (either by exon 19 deletion or punctual mutation in exon 21 known as L858R) show an increased amount and duration of EGFR activation compared with wild-type receptors [18]. Mutated EGFR can activate RAS/RAF/MEK/MAPK and phosphoinositide 3-kinase (PI3K)/AKT and STAT3/STAT5 pathways [19-21]. Beside the importance of EGFR on lung carcinogenesis, some other molecules have been described as molecular markers for prognosis and therapeutic targets. Gene amplification and mutations in the kinase domain of C-erbB2 (HER-2/neu), a member of EGFR family, have been identified in patients with lung adenocarcinomas with a frequency of less than 5% and 5 to 10% respectively, and its overexpression are involved in ~25% of NSCLC cases [22]. EGFR and HER-2 kinase domain mutations have similar associations with female sex, non-smoking status and Asian background in patients with adenocarcinoma [15,22]. RAS/RAF/MEK/MAPK pathway is involved in signaling downstream from EGFR leading the growth and tumor progression in NSCLC. Activating *kras* gene mutation occurs in ~30% of cases of NSCLC, mostly adenocarcinomas. KRAS mutations are localized in exon 12 (in 90% of patients) or exon 13, and they are smoking-related G→T transversion and nonsmoking-related G→A transition [23]. KRAS mutations appear to be an early event in smoking-related lung adenocarcinoma, representing a poor prognosis in these patients. Another promising predictive markers in NSCLC are BRAF [24] and the oncogenic fusion gene of EML4-ALK [25]. BRAF, an effector molecule of RAS pathway, is mutated in about 2% of adenocarcinomas that does not show *kras* gene mutations. While *eml4-alk* is present in 2% to 7% of NSCLC cases; essentially, this fusion gene is present in young patients with adenocarcinoma and no exposure to smoking [26] (Figure 2).

Some other molecules have been identified based on expression and genomic data such as MYC and Cyclin D1 which are amplified and over-expressed in 2.5–10% and 5% of NSCLC respectively, while BCL-2 over-expression is involved in ~25% of cases of NSCLC [8,16]. Recent data have shown that methylation of the promoter regions of genes is a common event in NSCLC, which contributes to oncogenes over-expression or tumor genes suppressors silenced. These epigenetic changes may be an early event in NSCLC, since that promoter region of p16 gene is frequently methylated in smokers and premalignant lesion of lung cancer [27]. PI3K-AKT-mTOR pathway is altered in NSCLC. AKT overexpression has been described in a subgroup of NSCLC tumors jointly with mutations or amplification of PIK3CA gene. These genomic modifications are related with enhanced activity of PI3-K pathway mainly in squamous cell carcinoma tumors [28]. On the other hand, tissues of smoker patients show higher levels of angiogenic factors such as VEGF. VEGF expression increases in relationship with tumoral grade, which in turn, correlates with increased microvessel density, development and poor prognosis of lung cancer. Tumoral angiogenesis and angiogenic factors are regulated by hypoxic inductor factor (HIF) 1α and 2α or through

oncogenes as *egfr*, *kras* and *p53* [29]. Genomics and proteomics tools have permitted the identification of molecules associated with a specific phenotype in cancer. Gene, microRNA and protein-expression signatures in lung cancer have allowed for the identification of molecules that show promise as biomarkers or therapeutic target for diagnosis, prognosis and therapeutic treatments [review 11,30]. The research focused on improving anti-tumor treatments in lung cancer has focused on genomic and proteomic study of tumors with specific genetic background, such as tumors with mutations in EGFR and KRAS. This molecular classification has had an influence on the response to biological therapies based on monoclonal antibodies (mAb) and tyrosine kinase inhibitors (TKIs) in lung cancer patients [15, 31-32], but now, we also know that the genetic background of lung tumors has an impact on the response to chemotherapy [33] and radiotherapy [34,35].

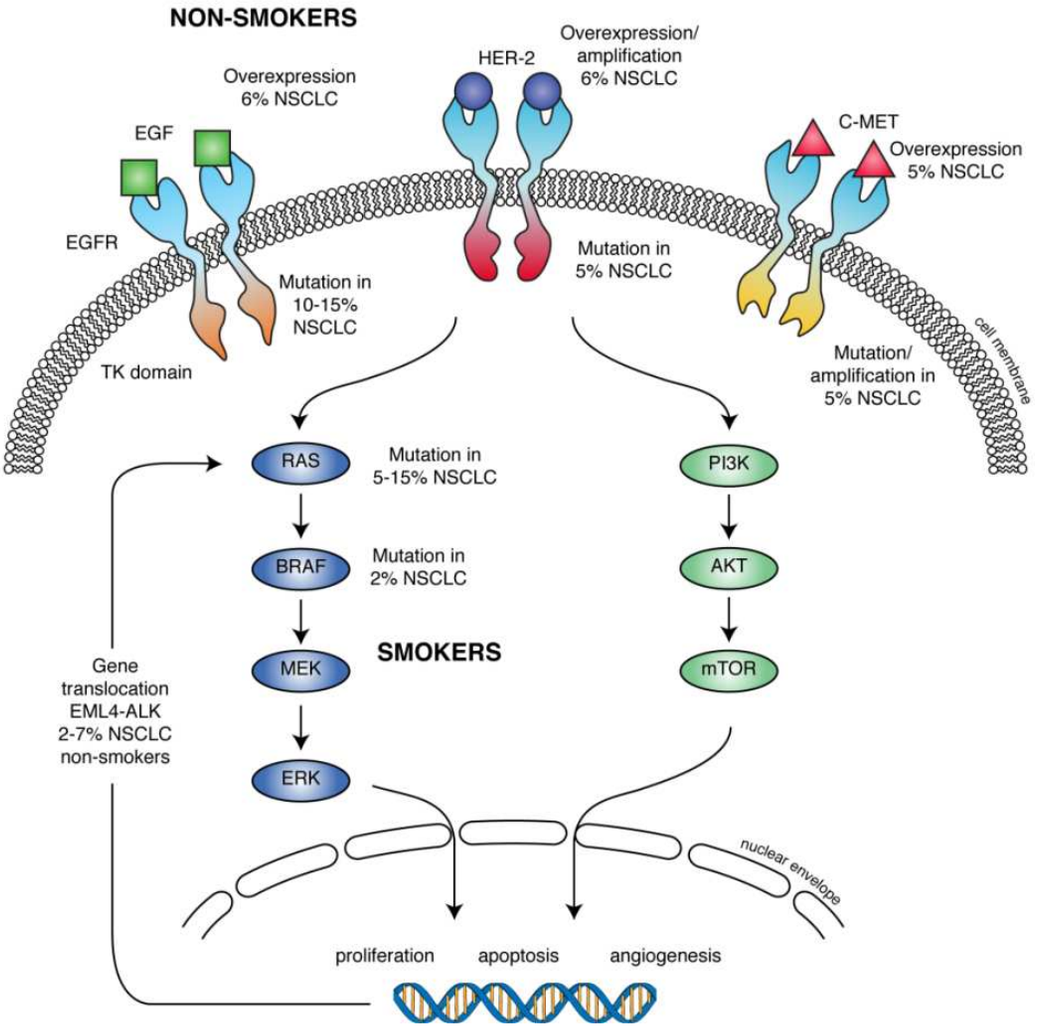

Figure 2. EGFR pathway in NSCLC. Mutations, amplification or overexpression of growth factors receptors such as EGFR, HER-2 and C-MET are most frequent in NSCLC tumors from non-smokers patients. All these genetic alterations have been observed commonly in adenocarcinomas, women and Asiatic ethnicity. EML4/ALK fusion gene is associated to NSCLC from young and non-smokers patients. KRAS mutations and signaling pathway depending to KRAS are most frequent in smoker patients. PI3K signaling pathway modifications are most frequently observed in squamous cell carcinomas.

4. Molecular and radiology therapies in lung cancer

4.1. Molecular therapy: Response and biological resistance

EGFR exhibits overexpression or aberrant activation in 50 to 90% of NSCLC. Mutations in EGFR allow sustained activation of EGF signaling for tumor cell survival, therefore, has been development targeted inhibitors for this molecule [16]. mAbs target the extracellular domain of EGFR and small molecules that inhibit intracellular EGFR tyrosine kinase domain function. In 2004, a significant advancement in the treatment of NSCLC was made following the observation that somatic mutations in the kinase domain of EGFR strongly correlated with sensitivity to EGFR TKIs [31, 32]. EGFR mutations are particularly prevalent in a patient subgroups with specific characteristics as adenocarcinoma histology, women, never smokers, and East Asian ethnicity [36]. This subgroup shows an exquisite sensitivity and marked tumor response to TKIs treatment. Despites the results obtained with biological therapies, there is a group of patients who do not respond to molecular therapy. Moreover, there is another group of patients with EGFR mutant lung cancer who initially respond to TKI treatment, but subsequently develop disease progression after a median of 10 to 14 months on treatment with biological therapy [37,38]. Hence, no optimal therapy thereafter has yet been established. Presumably, tumors do not respond because their molecular lesions are downstream of the therapeutic target [39]. Resistance to biologic therapy in NSCLC has been associated with EGFR exon-20 insertions [40] or a secondary T790M mutation [41], KRAS mutation [42], or amplification of the MET proto-oncogene [43,44], where MET is a transmembrane receptor with a tyrosine kinase domain, which activates signaling survival depending to PI3K and MAPK pathways. Of importance, Some reports showed that inhibition of MET signaling can restore sensitivity to TKIs [45]. HER-2 kinase domain mutations are associated with resistance to EGFR TKIs, but also with sensitivity to HER-2-targeted therapy [46].

Genomics data have provided information for developing targeted therapies in lung cancer patients based upon identification of cancer-specific vulnerabilities and set the stage for molecular biomarkers that provide information on clinical outcome and response to treatment. It has become clear that molecular-targeted cancer therapies can only reach their full potential through appropriate patient selection. In addition, there are now large clinical studies of lung cancer showing distinct chemotherapy and radiation responses. The majority of patients with lung cancer display advanced disease, these patients have obtained modest improvements in overall survival and quality of life through the use of systemic chemotherapy; however, the survival is still low, getting a median survival of 8 to 10 months [1]. Once recurred or metastasized, the disease is essentially incurable with survival rates at 5 years of less than 5%, and this has improved only marginally during the past 25 years [1]. The substantial genetic heterogeneity inherent to human cancers as an indicator of distinct phenotypes makes the identification of patients most likely to benefit from a given anticancer agent challenging. The description of molecules associated with resistance or sensitivity to cytotoxic treatments will improve personalized therapy for lung cancer. Radiotherapy, alone or in combination with surgery or chemotherapy, plays a critical role in

the management of lung cancer. More than 60% of lung cancer patients receive radiotherapy at least once during the course of their disease [47].

4.2. Role of EGFR pathways in resistance and sensibility to radiotherapy

NSCLC tumors exhibit a wide response spectrum to radiation therapy but the molecular basis for this responsiveness is unknown. Some patients with NSCLC have a good response to radiation therapy with long-term local control while others relapse even with high dose treatment [48]. Many factors are involved in biological process of lung damage induced by radiation. At the molecular level, it is established that ionizing radiation causes various types of cellular damage; the creation of DNA breaks represents the principal damage induced by direct action of ionizing radiation or indirect action provoked by reactive species oxygen (ROS). Inadequately repaired DNA breaks leads to loss of cell clonogenicity via the generation of lethal chromosomal aberrations or the direct induction of apoptosis [49]. In addition to DNA breaks, ROS rapidly triggers the production of cytokines, growth factors, and more ROS, ultimately leading to chronic oxidative stress, hypoxia and the nonhealing tissue response in the lung [50,51]. Tumor radioresistance, including intrinsic resistance before treatments and acquired resistance during radiotherapy, is one of the main obstacles for radiotherapy efficiency for NSCLC. Some of the most important mechanisms associated with radioresistance in cancer including checkpoint pathway, mismatch repair process, and DNA damage repair [52-54]. Accumulating evidence suggests that radioresistance is often correlated with some genes, such as p53 [55] and EGFR [56]. In this regard, targeting EGFR pathway activation radiosensitizes human cancer cells [57-59], suggesting that the presence of overexpressed or activated oncogenes such as EGFR or RAS may be a mechanism for increased cellular resistance to radiation. In some models, it has been demonstrated that EGFR/Ras/Raf/MEK/ERK signaling may be activated in response to radiation, promoting cancer cell survival and proliferation [52-54,60] (Figure 3).

Variations in NSCLC responses to radiotherapy alone or in combination with chemotherapy or biological therapy are most likely due in the majority of cases to the genetic and epigenetic constitution of tumors [61,62]. In NSCLC, EGFR and KRAS oncogenes play an important role as prognostic factors; therefore, their role in radioresistance has been documented [63]. NSCLC cell lines harboring EGFR with mutations in tyrosine kinase domain were many folds more sensitive to radiation compared to cell lines with wild type EGFR. Radiosensitivity of NSCLC cell lines with mutant EGFR and human bronchial epithelial cells stably expressing mutant forms of EGFR was attributed to delayed DNA repair kinetics, defective radiation-induced arrest during DNA synthesis or mitosis, and pronounced increases in apoptosis or the occurrence of micronuclei [63]. Apparently, mutant EGFR is unable to translocate into the nucleus, which hinders its interaction with DNA-dependent protein kinase (DNA-PK), which is a fundamental enzyme for repair radiation-induced double strand breaks [63]. Besides of the promising role of mutant EGFR in radiosensitivity, the effort by blocking EGFR pathway to induce better response to radiotherapy has been limited. Inhibition of the EGFR by TKI or mAb, has been shown to

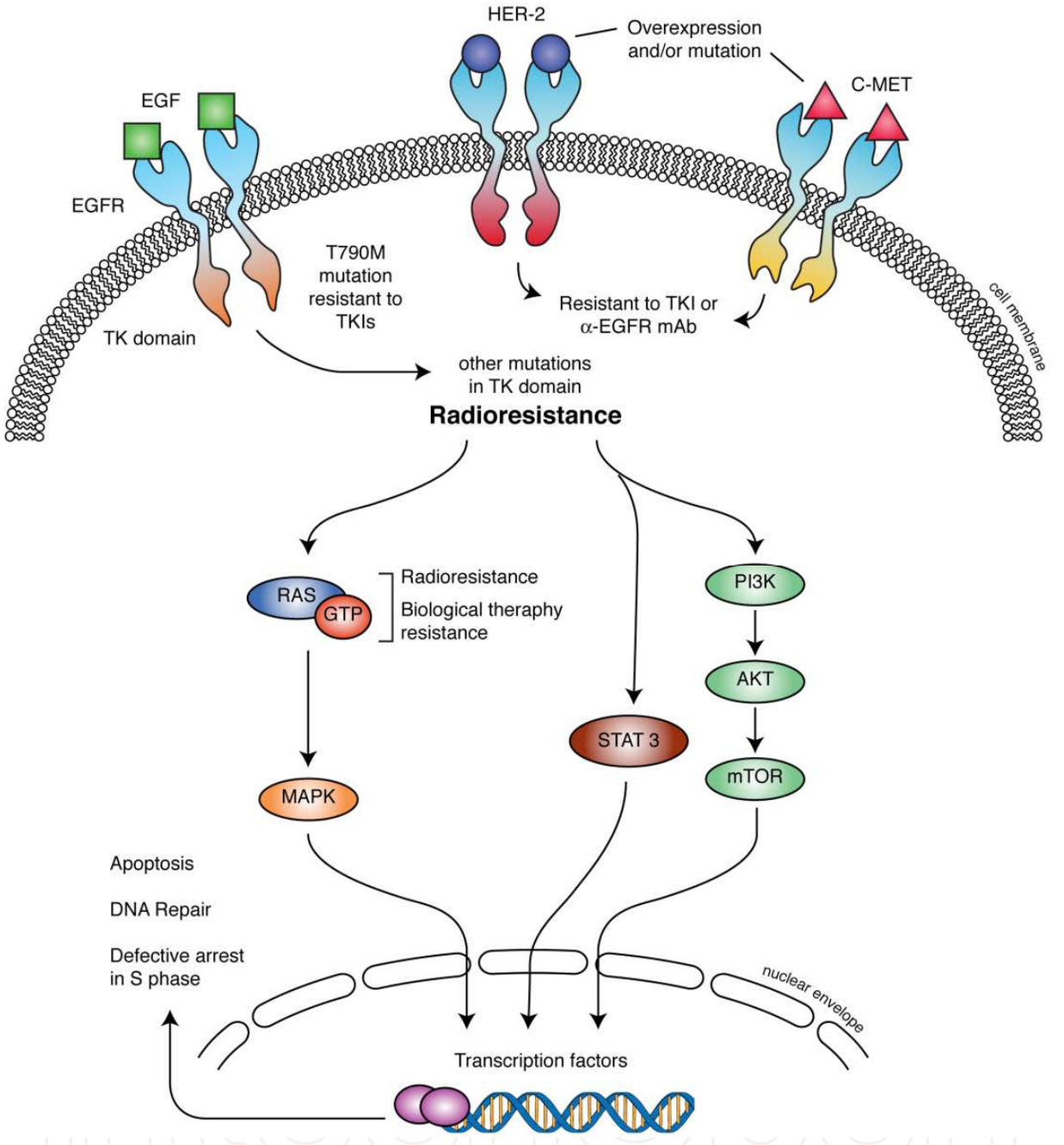

Figure 3. Role of EGFR pathway in radioresistance and radiosensibility in NSCLC. Aberrantly activation of EGFR pathways, including receptor mutations, KRAS activation, PI3K/AKT/mTOR pathway activation allows expression of specific genes for to regulate apoptosis, DNA repair, cell cycle and cell proliferation in order to get resistance to radiation.

radiosensitize a limited number of NSCLC cell lines *in vitro* and *in vivo* [34,35,63-65]. In NSCLC cell lines with wild-type or mutant p53, cell proliferation and clonogenic survival could be disturbed by senescence induced by EGFR inhibition and double strand breaks (DSB) produced by radiation. Apparently, radiosensitization by EGFR inhibitors is due to an increase in the levels of non-repairable DSB and disturbance of the MEK-ERK pathway [66]. Although a variety of signaling pathways downstream of EGFR have been implicated in

radioresistance, including PI3K-AKT, MEK-ERK, and PLC-PKC [67-69], no evidence of a common molecular pathway of radiosensitization, and cellular mechanisms by which EGFR TKI and mAb may cause radiosensitization remained largely elusive. Activating KRAS mutations is a marker for worse prognosis in NSCLC [23, 26]. Sun *et al*. evaluated whether the presence of mutation could be a potential factor for radioresistance. The results showed a reduced level of apoptosis in response to radiation in lung cancer cell line HCC2429 transfected with mutant KRAS 12V (mutation in codon 12). The authors suggested that phosphorylation of ERK could contribute to the low levels of apoptosis induced by radiotherapy in mutated KRAS lung cancer cells. This work suggests that KRAS mutation status is one potential factor associated with increased resistance to radiation-induced apoptosis in lung cancer cells [70]. The same group has recently shown that the specific inhibition of JAK2 by the novel molecule TG101209, induces radiosensibility through inhibition of phosphorylation of STAT3 and reduced expression of survivin in HCC2429 lung cancer cells. Moreover, the inhibition of survivin by treatment with TG101209 in experiments *in vivo*, was related to increased apoptosis, reducing tumor proliferation and vascular density [70]. Lu *et al*. demonstrated that overexpression of survivin leads to radioresistance in H460 lung cancer cells by inhibiting apoptosis and promoting cell survival, however, when survivin is inhibited by antisense oligonucleotides the cytotoxic effect of radiation is enhanced [71]. These results suggested that survivin might be a molecular marker for prognostic response to radiotherapy in NSCLC. While inhibition of survivin expression in HCC2429 and H460 cells were related to radiosensibility, both cell lines showed different apoptosis levels which were related to radioresistance depending on KRAS mutation status.

5. Lung cancer radiogenomics

Radiotherapy has played a key role in the control of tumor growth in many cancer patients, including lung cancer. Studies that originated more than 40 years ago [72,73] have indicated that tumors respond to radiotherapy by initiating a process called accelerated repopulation. In this process, the few surviving cells that escaped death after exposure to radiotherapy or chemotherapy can rapidly repopulate the badly damaged tumor by proliferating at a markedly faster pace. This phenomenon suggested that tumoral heterogeneity permits a cell population in the tumor to have advantages to avoid cell death induced by radiation. Cellular senescence, DNA repair and cell cycle checkpoint are cellular mechanisms that influence the resistance to radiotherapy. However, the molecular mechanisms that regulate the radioresistance phenotype have not been clear in cancer. For this reason, some research groups have focused in the study of biological models to obtain genomic and proteomic signatures in order to find genes and proteins that could predict radiosensitivity or radioresistance in lung tumors (Table 1). Although such researches have contributed to a partial understanding of the mechanisms underlying cellular radioresistance, the comprehensive functional mechanisms remain largely elusive. This may be quite reasonable since the mechanisms of radioresistance are a complex multigene interaction. In this sense, Torres-Roca *et al*. [74] in 2005, hypothesized that a radiation sensitivity classifier or predictor

could be developed based on gene expression profiles derived from DNA microarrays. This hypothesis was based in the fact of three main biological mechanisms partially correlated with clinical failure to radiotherapy, which are: hypoxia, intrinsic radiosensitivity and proliferation. These mechanisms, in turn, are handled by changes in gene expression.

Radiosensibility	
c-Jun*	[75]
HDAC-1	
RELA (p65 subunit NFkB	
PKC-beta	
Sumo1	
c-Ab1	
STAT1	
AR	
CDK1	
IRF1	
Innate Radioresistance	
Up-regulated in basal condition	[76]
XRCC5	
ERCC5	
ERCC1	
RAD9A	
ERCC4	
Up-regulated after radiation	[76]
MDM2*	
BCL-2	
PKC-2	
PIM2	
Acquired Radioresistance	
Up-regulated	[77]
DDB2	
LOX	
CDH2	
CR4AB	
Livin α*	[79]
Down-regulated	[77]
GBP-1	
CD83	
TNNC1	
TP53I3*	[78]

* Validated genes

Table 1. Genes associated to radiation response in NSCLC from genomics data

The authors developed a radiation classifier to calculate the radiosensitivity of tumor cell lines based on basal gene expression profiles obtained from the literature. They predicted the survival fraction to 2 Gy (SF2) value in 22 of 35 cell lines from the National Cancer Institute, a result significantly different from chance (P = 0.0002). In their approach, radiation sensitivity as a continuous variable, significance analysis of microarrays is used for gene selection, and a multivariate linear regression model is used for radiosensitivity prediction. In gene selection, they identified three novel genes: RbAp48, RGS19, and R5PIA, whose expression values correlated with radiation sensitivity. Exogenous overexpression of RbAp48 into three cancer cell lines (HS-578T, MALME-3M, and MDA-MB-231) induced radiosensitization (1.5- to 2-fold), moreover, higher proportion of transfected cells with RbAp48 were in G2-M phase of the cell cycle (27% versus 5%). Finally, RbAp48 overexpression is correlated with dephosphorylation of Akt, suggesting that RbAp48 might be exerting its effect radiosensitized by antagonizing the Ras pathway, but it could also do so through PI3K. The authors establish that radiation sensitivity can be predicted based on gene expression profiles and they introduce a genomic approach to the identification of novel molecular markers of radiation sensitivity. Despite of results in different tumor cell lines, this work included only four NSCLC cell lines and they were able to predict correct SF2 values for only two of them [74]. So, the study should be performed on a broader panel of NSCLC cell lines. In lung cancer, multiple studies have identified a wide array of genetic and epigenetic alterations, including mutations in DNA sequence, DNA copy number changes, aberrant DNA promoter methylation, changes in mRNA, microRNAs and protein expression [8], revealing many potential determinants and signaling pathways governing lung tumorigenesis and progression. Gene expression profiling analysis allows for an increase in the understanding of the molecular mechanisms and pathways that involve radioresistance. Thus, the strategy followed by Torres-Roca and collaborators can be applied to gene expression data reported in lung cancer, in order to identify new molecular targets for radiotherapy response. In this sense, we know that the response of tumor cells to radiation is accompanied by complex changes in the gene expression pattern. Based on mRNA expression profiles and systems-biology approach, Eschrich *et al*. [75] applied a linear regression algorithm that integrates gene expression with biological variables, including RAS and p53 status (mut/wt), and tissue of origin, with the aim of understanding radiosensitivity and identifying radiation specific markers. The modeling of radiosensitivity represented for the survival fraction at 2 Gy of 48 human cancer cell lines reported a direct correlation between gene expression and radiosensitivity of the lung cancer cell lines. The authors developed a model that classified four different clusters of genes that were markers for radiosensitivity. They identified 10 gene networks comprised by c-Jun, HDAC1, RELA (p65 subunit of NFKB), PKC-beta, SUMO-1, c-Abl, STAT1, AR, CDK1 and IRF1. Interestingly, RAS was a dominant variable in the analysis, as was the tissue of origin (lung), and their interaction with gene expression but not with p53. Moreover, when they knocked-down c-Jun in eight different cancer cell lines (lung, colon and breast cancer) there was an overall trend toward radioresistance, predominantly in lung cancers, but not in breast or colon cancers, implying that the origin of the tissue was important [75].

A problem in radiogenomics research is the difficulty to determine what fraction of the tumor cell population is radioresistant after a course of radiotherapy. For understand the radiation-mediated changes in gene expression that might result in different responses to radiation, Guo W *et al*. in 2005 [76] designed an oligonucleotide microarray to analyze the expression of 143 genes in lung cancer cell lines that differed in radiosensitivity. In the radioresistant A549 cells, 8 genes were significantly up-regulated and 10 genes were down-regulated compared to radiosensitivity NCI-H446 cells. When the lung cancer cell lines were irradiated with 5Gy of γ rays, they identified genes showing altered expression and potential candidate genes that might confer radioresistance. In A549 cells, 19 up-regulated and 3 down-regulated genes, and 8 up-regulated and 18 down-regulated genes were found 6 and 24 h after irradiation, respectively. In NCI-H446 cells, the expression of 9 up-regulated and 8 down-regulated genes, and 8 up-regulated and 12 down-regulated genes was altered 6 and 24 h after irradiation, respectively. They found that MDM2, BCL2, PKCZ and PIM2 expression levels were increased in A549 cells and decreased in NCI-H446 cells after irradiation. Whereas, XRCC5, ERCC5, ERCC1, RAD9A, ERCC4 and the gene encoding DNA-PK were found to be increased to a higher level in A549 cells than in NCI-H446 cells. Inhibition of MDM2 by an antisense oligonucleotide in A549 cells resulted in increased radiosensitivity. The authors demonstrate the possibility that a group of genes involved in DNA repair, regulation of the cell cycle, cell proliferation and apoptosis are responsible for the different endogenous radioresistance between these two lung cancer cell lines [76]. To continue searching for new molecular evidences for radioresistance, Qing-Yong *et al*. in 2008 identified gene expression profiles in lung adenocarcinoma cell line Anip973 and obtained radioresistant phenotype cells (Anip973R). Expression profiles were obtained by oligonucleotide microarrays consisting of 21,522 human genes, while radioresistant cells Anip973R were obtained by fractionated ionizing radiation treatment of 4 Gy until a total dose of 60 Gy. In Anip973R cells, the authors reported 59 up-regulated genes associated with DNA damage repair (DDB2), extracellular matrix (LOX), cell adhesion (CDH2), and apoptosis (CRYAB); and 43 down-regulated genes associated with angiogenesis (GBP-1), immune response (CD83), and calcium signaling pathway (TNNC1). Validation of the selected eleven genes, including CD24, DDB2, IGFBP3, LOX, CDH2, CRYAB, PROCR, ANXA1 DCN, GBP-1 and CD83 by Q-RT-PCR was consistent with microarray analysis [77]. In 2010, Lee *et al*.. analyzed expression profiles of H460 NSCLC radiosensitive cell lines and their radioresistant counterpart (H460R) cells established by fractionated irradiation. By utilizing a cDNA microarray, they identified 1,463 genes altered more than 1.5-fold in H460R compared with parental H460. Tumor protein p53-inducible protein 3 (TP53I3) gene was significantly down-regulated in radioresistant H460R cells predicting a link to p53-dependent cell death signaling. Interestingly, mRNA expression of TP53I3 differed in X-ray–irradiated H460 and H460R cells, and overexpression of TP53I3 significantly affected the cellular radiosensitivity of H460R cells [78]. These works showed that fractionated ionizing radiation can lead to the development of acquired radiation resistance across altered gene profiles. Genomic profile using *in vivo* models of radioresistance may provide new insights into mechanisms underlying the promotion of clinical resistance for NSCLC. Some other

researches have been focused in describing specific molecules that revert the radioresistant phenotype. It is well known that there is a large amount of cell death during cytotoxic cancer therapy such as radiotherapy; therefore, radioresistance is associated with deregulation of apoptosis proteins. Sun *et al*. in 2011 reported the role of livin in radioresistance of lung adenocarcinomas cell lines A549 and SPC-A1. Livin is a IAPs family member whose expression is related with apoptosis inhibition, in some studies, it has been suggested that livin may be of clinical significance [79]. This work showed that A549 lung adenocarcinoma cells do not express livin in basal condition, but it is expressed after cells were irradiated. Moreover, gene silencing of livin by siRNA in SPC-A1 lung cell line induced a remarkable sensibility to radiation. Additionally, the authors showed that the isoform livin α had more impact on radioresistance that livin β had. These results suggested that livin expression in lung adenocarcinoma cells could be a radioresistance mechanism through down-regulation of apoptosis. The cytotoxicity of oncological therapies is highly dependent on the cell cycle phase. G2/M phase is the one most sensitive to ionizing radiation. A work published in 2010 determined that arresting time on G2/M cell cycle phase is different between NSCLC cell lines sensitive and resistance to ionizing radiation. Radiosensitive H460 NSCLC cell line showed a significant G2/M arrest after 12 h of irradiation with 5 Gy of γ rays, while radioresistant A549 cell lines showed a significant G2/M arrest after 12 h of radiation. Interestingly, the arrest in A549 completely disappeared after 24 h of radiation. The arrest on G2/M correlated with higher methylated CpG sites of PTEN gene and consequently, reducing expression of the protein. PTEN negatively regulate pAKT which regulate negatively to p53. Therefore, radioresistance of A549 may depend to over activation of p53 signaling pathways. Epigenetic gene modification may be a way for regulating genes that participate in radiation response [80]. Signal transduction pathways depending to STAT have been explored. In A549 and SK-MES-1 cells, the exogenous over-expression of STAT3 was evaluated for its role in radioresponse. STAT3 over-expression enhanced the sensitivity to ionizing radiation *in vitro* and *in vivo*. Apparently, the radiosensibility may induce through STAT3-dependent inhibition of growth and induction of apoptosis [81]. These works showed that the regulation of signaling molecules that control apoptosis, cell growth and cell cycle has an important role in positive or negative radiation response.

6. Proteomics of radiation response in lung cancer

Despite proteomics being useful to find molecular markers associated to lung cancer cells [82], in radiation resistance research there are very few studies focused on applying proteomics to find new markers associated to radiotherapy response in lung cancer. Recently, Wei R *et al*. [83] in 2012 evaluated the multidrug resistance (MDR) effect on the radioresistance (RDR) in human lung adenocarcinoma cell lines and tissues. In this work, the authors screen MDR- and RDR-related proteins after irradiation of A549 and A549/DDP (resistant to cisplatin) human lung adenocarcinoma cells. The cell lines were analyzed by colony-forming assay and flow cytometry. Two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-

TOF–MS) were utilized to identify differentially expressed proteins between irradiated A549 and A549/DDP. The SF2 value increased and the mean percentage of G2 phase and apoptosis rate decreased significantly in A549/DDP cells compared with A549 cells. Forty spots were found, and among them, 27 were identified through proteomics. Four up-regulated proteins (HSPB1, Vimentin, Cofilin-1, and Annexin A4) were confirmed by Western blot in MDR cells as compared with non-MDR cells. Immunohistochemistry showed that they were also over-expressed in MDR tissues compared with non-MDR counterparts of human lung adenocarcinomas. These results proved that the MDR in lung adenocarcinoma cells and tissues increased the radioresistance. HSPB1, Vimentin, Cofilin-1, and Annexin A4 are potential biomarkers for predicting lung adenocarcinomas response to chemo- and radiotherapy, as well as novel targets for treatment of lung adenocarcinomas [83].

7. Conclusion

One of the most important problems in lung oncology is lack of suitable biomarkers as therapeutic targets or the absence of predictors of therapy response. The genetic heterogeneity of the lung tumors influences the initial molecular resistance to therapies, but also in the development of resistance during treatment. The molecular mechanisms that influence the resistance to biological or radiological treatments, referring to the resistance mechanisms occurring naturally because of the carcinogenic process, or those developing as a result of evolutionary pressure that tumor cells undergoing during the treatment administration, is a barrier that has not been fully elucidated. With current genomics and proteomics studies in lung cancer focused on solving the mystery of therapeutic resistance, it has been possible to identify molecules that may serve as prognostic markers of response to radiological and molecular therapy resistance. Genes and proteins that regulate cell proliferation and survival, including signaling molecules and transcription factors such as KRAS, BRAF, PI3K, MAPK, mTOR, JAK2, STAT, survivin and others have demonstrated to be part of the molecular machinery that regulates therapeutic resistance. Moreover, gene and protein expression profiling of lung cancer has focused specifically on searching predictive markers to radiotherapy. Some studies have generated data on molecules involved in radioresistance or radiosensitivity either natural or acquired. Using therapeutic doses of radiation in *in vitro* models, it have described proteins implicated in DNA repair, cell cycle checkpoint and cell death. Mutations in EGFR pathway have played an important role as therapeutic targets for development of new therapies, moreover, mutations in this pathway represent a mechanism of radioresistance, suggesting that aberrant activation of EGFR pathway, including activated mutations in EGFR and KRAS might be an innate radioresistance mechanism in NSCLC. Despite advances in proteomics and radiogenomics in lung cancer, an enormous need to implement *in vivo* and clinical models for identification of effective biomarkers predictive in radio-oncology has also became evident. This is currently a promising field of cancer research in which genomics, tumor molecular biology and clinical experience interact to

achieve more effective combination therapies adjusted to the patient profile. Understanding the mechanisms of radioresistance of cells from solid tumors is of prime importance for further improvement of radiotherapy.

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