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The Genomics of Lung Adenocarcinoma: Opportunities for Targeted Therapies

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Abstract

Standard cytotoxic chemotherapy is effective for some cancers, but for many others, available treatments offer only a limited survival benefit. Lung adenocarcinoma is one such cancer, responsible for approximately half of lung cancer deaths each year. Development of targeted therapies is thought to hold the most promise for successfully treating this disease, but a targeted approach is dependent on understanding the genomic state of the tumor cells. Exon-directed sequencing of large numbers of lung adenocarcinoma tumor samples has provided an initial low-resolution image of the somatic mutation profile of these tumors. Such cancer sequencing studies have confirmed the high frequency of *TP53* and *KRAS* mutations in lung adenocarcinoma, have found inactivating mutations in known tumor suppressor genes not previously associated with lung adenocarcinoma, and have identified oncogenic mutations of *EGFR* upon which the first targeted therapy for lung adenocarcinoma patients was based. Additional candidate oncogenes await functional validation. It is anticipated that upcoming whole-exome and whole-genome lung adenocarcinoma sequencing experiments will reveal a more detailed landscape of somatic mutations that can be exploited for therapeutic purposes.

Keywords: lung adenocarcinoma, EGFR, cancer sequencing, targeted therapy

Introduction

Lung cancer is the leading cause of cancer death in the United States and worldwide, accounting for over 150,000 deaths annually in the United States alone.[1] The overall 5-year survival rate for lung cancer is only 16%, largely driven by the high frequency of late diagnosis, resulting in nonresectable tumors.[1] Lung cancer can be histologically subclassified into 4 major categories: lung adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, comprising non–small cell lung cancer (NSCLC), and small cell carcinoma of the lung.[2] Lung adenocarcinoma, an epithelial cancer of glandular origin, is the most prevalent of these lung cancer diagnoses, including in never-smokers.[3]

The abysmal survival rate for lung adenocarcinoma reflects the inadequacy of traditional cytotoxic chemotherapy for this disease; therapies targeted to tumor cell vulnerabilities instead hold the most promise for the future. Somatic mutations that activate oncogenes frequently result in tumor cell dependency on the altered oncogene products,[4,5] a property exploited by the prototypical targeted therapy, imatinib mesylate. Imatinib mesylate inhibits the Bcr-Abl fusion protein, resulting from a recurrent translocation in chronic myelogenous leukemia.[6] Imatinib additionally inhibits activated forms of the related tyrosine kinases KIT and PDGFRA and has been successfully used in gastrointestinal stromal tumors harboring mutations in these genes.[7] The identification of recurring oncogenic lesions in lung adenocarcinoma upon which the tumor cell depends for survival may therefore lead to novel lung cancer therapies.

A large-scale exon-directed sequencing experiment, the Tumor Sequencing Project (TSP), was undertaken in order to begin to address the question of recurring somatic mutations in lung adenocarcinoma. In this experiment, all coding exons of 623 cancer-related genes were sequenced in 188 tumor/normal DNA pairs, resulting in the identification of 1,013 nonsynonymous somatic mutations.[8] Statistical analysis indicated that 26 genes were mutated at a rate significantly higher than the background mutation rate, indicative of positive selection (Fig. 1). These 26 significantly mutated genes included several well-characterized oncogenes and tumor suppressor genes already known to be involved in lung cancer, *KRAS*, *TP53*, *STK11*, *EGFR*, and *CDKN2A*. In addition, a number of significantly mutated genes not previously reported in lung adenocarcinoma were identified, including known tumor suppressor genes and several tyrosine kinase genes that represent candidate oncogenes pending functional validation.

Here, I describe the state of knowledge of the genomics of lung adenocarcinoma as advanced by the TSP experiment with special attention to therapeutic implications. The upcoming wave of whole-exome and whole-genome lung adenocarcinoma sequencing results, facilitated by next-generation sequencing technologies, will likely

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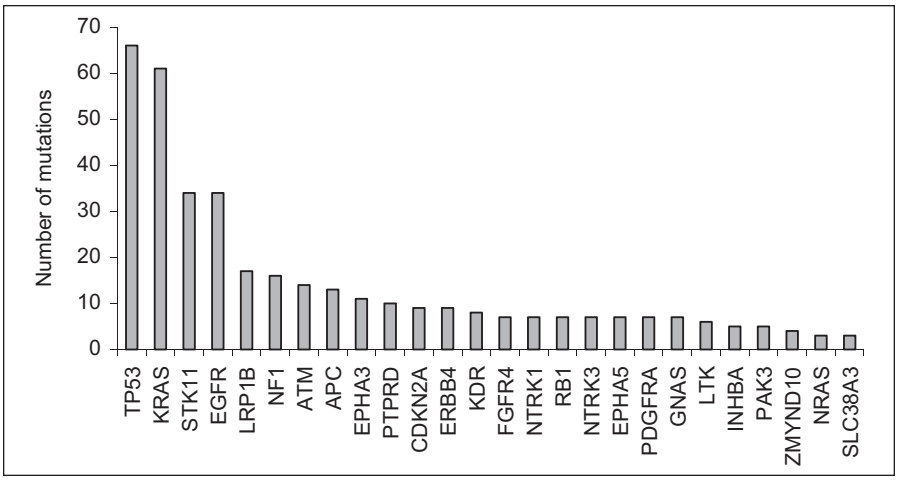

Figure 1. Significantly mutated genes from the lung adenocarcinoma Tumor Sequencing Project. Adapted from Ding *et al*.[8]

revolutionize our understanding of the genomics of this disease once more.

Mutually Exclusive Oncogenic Alterations

Somatic alterations of 5 lung adenocarcinoma oncogenes, *KRAS*, *EGFR*, *ALK*, *ERBB2*, and *BRAF*, are interestingly mutually exclusive and are represented in over 50% of lung adenocarcinomas.[9,10] In fact, patients with mutations in these 5 genes may account for up to 90% of Asian never-smokers with the disease.[11] The ability to therapeutically inhibit the functions of these 5 altered genes would therefore represent significant progress in the battle against lung cancer.

KRAS

Mutations in *KRAS*, the most frequently mutated oncogene in lung adenocarcinoma described to date, have been known for some time.[12,13] *KRAS* encodes a low molecular weight GTPase that signals through RAF and ERK when GTP bound.[14,15] Similar to *KRAS* mutations found in other tumor types, mutations that replace Gly 12 with any one of several other amino acids are especially common, with substitutions at Gly 13 and Gln 61 also observed, at a combined frequency of 32%.[8] These mutations are activating and oncogenic, causing a reduction in GTPase activity and an increase in GTP-bound protein, resulting in increased mitogenic signaling through RAF.[12,14,15]

Despite the high frequency of *KRAS* mutations in lung adenocarcinoma and other cancers, it has proven difficult to exploit mutant KRAS as a therapeutic target. Early efforts were aimed at blocking C-terminal farnesylation, a post-translational modification required for protein activity.[16] Phase III clinical trials of farnesyl transferase inhibitors in solid tumors did not show any statistically significant overall survival benefit, possibly because of the alternate KRAS prenylation activity of geranylgeranyl transferase I, resulting in continued membrane association in the presence of farnesyl transferase inhibitors.[16,17]

Inhibition of downstream signaling proteins RAF and MEK might also be expected to inhibit growth of tumors cells harboring *KRAS* mutations, but this approach has been largely unsuccessful as well. Although a combination of PI3K and MEK inhibition can reverse lung adenocarcinomas in transgenic mice driven by *KRAS* G12D,[18] phase II trials of MEK inhibitors as single agents in unselected NSCLC patients have shown a lack of efficacy thus far.[19-21] Treatment with sorafenib, a small molecule inhibitor of BRAF and CRAF and several other kinases, resulted in stable disease for 59% of unselected NSCLC patients in a phase II trial, but no responses were observed.[22] Moreover, preclinical studies demonstrated that treatment of *KRAS* mutant cells with a specific BRAF inhibitor paradoxically activated the RAF-MEK-ERK pathway in a CRAF-dependent manner, indicating that BRAF inhibitors are not suitable for use in tumor cells harboring *KRAS* mutations.[23-25]

One current area of active research in targeting lung adenocarcinoma cells harboring *KRAS* mutations involves a synthetic lethal approach,[26] whereby inhibition of a second protein causes cell death only in *KRAS* mutant cells. Interestingly, several RNA-interference synthetic lethal screens have recently been completed in *KRAS* mutant and wild-type cell lines, identifying the kinases STK33, TBK1, and PLK1 as possible synthetic lethal therapeutic targets.[27-29] Additional experiments in tumor cell lines dependent on mutant *KRAS* for survival or mouse models of lung cancer driven by mutant *KRAS* pinpointed inhibition or knockdown of NFκB, CDK4, SYK, integrin β6, and RON as synthetic lethal with *KRAS* mutation.[30-32] Whether any of these synthetic lethal interactions translate to a lung cancer therapy remains to be determined.

EGFR

Recurring mutations of the epidermal growth factor receptor (*EGFR*) tyrosine kinase were first reported in lung adenocarcinoma in 2004 in about 10% of Western patients and over 40% of East Asian patients,[33-35] although the biology of this ethnic disparity remains unclear. Mutations were initially identified in 3 kinase domain exons, encoding G719S or G719C in exon 18, small in-frame deletions in exon 19, and L858R or L861Q in exon 21. The observed mutations were determined to be constitutively activating and oncogenic[36] and importantly correlated with patient response to gefitinib and erlotinib, small molecule inhibitors

of EGFR.[33-35] By contrast, oncogenic small in-frame insertions of exon 20 were subsequently discovered in lung adenocarcinoma patients[37-39]; these EGFR mutants were not sensitive to gefitinib or erlotinib and thus comprised a class of primary resistance mutations in lung adenocarcinoma.[36,40]

There was some early controversy regarding whether *EGFR* mutations were truly predictive of gefitinib and erlotinib response, possibly in part because of the confounding effect of the difficulty of somatic mutation detection in stromally contaminated tumors as well as the shortage of evaluable tissue in some trials.[41] However, a recent series of phase III clinical trials in Asian patients confirmed a survival benefit of gefitinib over standard chemotherapy as a first-line agent for lung cancer patients who harbor *EGFR* mutations.[42-44] Mutant EGFR is thus a proven therapeutic target in lung adenocarcinoma.

Although patients harboring EGFR mutations in exons 18, 19, and 21 respond well to gefitinib and erlotinib, the response is not durable, and patients relapse after about a year of treatment.[41] The most frequent mechanism by which patients develop resistance to gefitinib or erlotinib treatment is acquisition of a second-site resistance mutation in exon 20 of *EGFR*, encoding T790M, which occurs in about 50% of relapsed patients.[45,46] This mutant is analogous to the ABL T315I "gatekeeper" residue substitution that occurs in chronic myeloid leukemia patients in blast crisis phase following an initial response to imatinib treatment.[47] The EGFR T790M mutation in particular has also been shown to decrease EGFR affinity for gefitinib in the context of L858R mutation via increased affinity for ATP.[48] Interestingly, rare germline mutations encoding EGFR T790M appear to cause inherited susceptibility to lung cancer, often accompanied by activating somatic mutations in *EGFR* exons 18, 19, and 21.[49,50]

Gefitinib and erlotinib are thus ineffective against the T790M acquired resistance mutation. However, a second generation of irreversible EGFR inhibitors that covalently modify the protein has recently been developed. Preclinical activity of several of these compounds in L858R-T790M model systems looked promising, especially in combination with rapamycin,[51,52] but clinical benefit has yet to be demonstrated. The recent discovery of an anilinopyrimidine-based small molecule that preferentially binds and inhibits EGFR T790M over wild-type has also generated much excitement.[53]

A second mechanism for the development of resistance to gefitinib, amplification of the receptor tyrosine kinase *MET*, has been identified in approximately 20% of patients[54] but can preexist prior to treatment and is not mutually exclusive with T790M mutation.[55,56] Resistant cells harboring *MET* amplification maintained upregulated PI3K signaling in an *ERBB3*-dependent manner even in the presence of gefitinib.[54] *In vitro* studies indicate that treatment with a combination of gefitinib and a MET inhibitor may circumvent resistance to gefitinib mediated by *MET* amplification, with the caveat that additional alterations resistant to the combination of both inhibitors, such as MET Y1230H, may also arise.[54,57]

ALK

Translocations between the receptor tyrosine kinase gene *ALK* and echinoderm microtubule-associated protein 4, *EML4*, resulting in the fusion protein EML4-ALK, were described in lung adenocarcinoma in 2007.[58] Although originally reported in 7% of NSCLC patient samples tested, the actual frequency may be closer to 4%. NIH-3T3 cells expressing the *EML4-ALK* variant formed tumors when injected into nude mice, confirming the oncogenic nature of the translocation.[58]

Because recurring *NPM-ALK* translocations had already been described in anaplastic large cell lymphoma,[59] efforts were under way to develop ALK inhibitors for this disease, facilitating rapid testing of ALK inhibitors in lung adenocarcinoma preclinical models and clinical trials. Although the ALK inhibitor TAE684 was cytotoxic in only 1 of 3 lung adenocarcinoma cell lines harboring an *EML4-ALK* translocation, the same small molecule efficiently caused tumor regression in transgenic mouse models of *EML4-ALK*–driven disease.[60,61] Importantly, data from an early clinical trial of crizotinib, a dual ALK and MET inhibitor, in NSCLC patients with *EML4-ALK* translocations look promising.[62]

Similar to *EGFR* mutant lung adenocarcinoma patients treated with gefitinib or erlotinib, patients who develop resistance to crizotinib treatment have been identified. Reported acquired resistance alleles of *EML4-ALK* encode ALK C1156Y, L1196M, and F1174L[63,64]; interestingly, ALK F1174L was also found to be a driver oncoprotein in neuroblastoma patients naïve of ALK inhibitor treatment.[65-68]

ERBB2

Somatic mutations of *ERBB2* in lung adenocarcinoma were first described in the same year as the *EGFR* mutations, albeit at lower frequency, approximately 2% to 4%.[69,70] These mutations are typically small in-frame insertions in exon 20 of the kinase domain, analogous to the primary resistance mutations of *EGFR* in the paralogous exon 20. ERBB2 is a receptor tyrosine kinase that does not bind any known ligand but homodimerizes or heterodimerizes with the highly related EGFR and other members of the ERBB family, ERBB3 and ERBB4, to activate downstream signaling pathways.[71] These mutations are activating and oncogenic in cell-based transformation assays and respond *in vitro* to the irreversible inhibitors of EGFR that also bind and inhibit ERBB2.[51,72-74] Again, whether these inhibitors are clinically effective against kinase domain mutants of ERBB2 found in lung adenocarcinoma remains to be demonstrated. The therapeutic antibody

trastuzumab, developed against the wild-type receptor for use in wild-type *ERBB2*-amplified breast cancer, does not look promising in preclinical models of mutant ERBB2.[74-76]

Oncogenic and drug-sensitive mutations of the extracellular domain of *EGFR* have been described in glioblastoma,[77,78] raising the possibility that extracellular domain mutations of *ERBB2* may also be found in cancer patients. In fact, a mutation encoding ERBB2 S310F was reported in the lung adenocarcinoma TSP.[8] This mutation, although not frequent in lung adenocarcinoma, has been found in other cancers as well and is oncogenic and sensitive to irreversible inhibitors of EGFR/ERBB2 *in vitro* (H. Greulich, unpublished data), raising the necessity of looking beyond the kinase domain of *ERBB2* for clinically relevant activating somatic mutations.

BRAF

Mutations of the serine/threonine kinase gene *BRAF* have been found at low frequency, about 2%, in lung adenocarcinoma. These mutations, first reported in 2002,[79,80] tend to occur in exons 11 and 15 of the kinase domain; however, the V600E mutations frequently found in melanoma and other cancers are rare in lung adenocarcinoma. Although the precise role of these mutations in the development of lung adenocarcinoma remains somewhat enigmatic, there is evidence of gain of function for at least some of the observed alleles.[81]

Although V600E mutant melanoma has recently been successfully targeted with an inhibitor of BRAF, PLX4032, this inhibitor does not have activity against other BRAF mutants, including those more commonly found in lung adenocarcinoma.[82,83] Testing of other BRAF inhibitors in *BRAF* mutant lung cancer has not been reported. A more promising therapeutic avenue may be MEK inhibition, which inhibits growth of lung adenocarcinoma cell lines harboring *BRAF* mutations.[84] Several such agents have failed to show efficacy in unselected NSCLC patients,[19-21] but given the low frequency of *BRAF* mutation in lung adenocarcinoma, a trial targeting only lung cancer patients with *BRAF* mutations may be required to uncover any possible therapeutic effect.

Nonmutually Exclusive Oncogenic Alterations

Two other oncogenes, *NRAS* and *PIK3CA*, exhibit recurring mutations in lung adenocarcinoma but are not mutually exclusive with the 5 described above. These 2 lung cancer genes will be discussed below along with rare known oncogenic mutations in other genes uncovered by the lung adenocarcinoma TSP and other sequencing efforts.

PIK3CA

A somatic mutation of *PIK3CA*, encoding the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3K), was first reported in lung cancer in 2004, along with similar mutations at a much higher frequency in colorectal carcinoma.[85] In light of subsequent reports, the overall mutation frequency appears to be 1% to 2%, and mutations cluster in the helical and kinase domains as is the case for *PIK3CA* mutations in other cancers.[86-88] Many of the observed mutations have been shown to be activating and oncogenic in transformation assays and to increase invasiveness in xenograft models,[89,90] and PI3K inhibition did reverse lung tumorigenesis in transgenic mouse models driven by *PIK3CA* H1047R,[18] but RNA interference and inhibitor experiments have not yet demonstrated a convincing response in adenocarcinoma cell lines. This indicates that inhibition of PI3K may not be sufficient for tumor therapy, even in patients harboring activating mutations, possibly because of the coexpression of additional mutationally activated oncogenes.

NRAS

Like *KRAS*, *NRAS* encodes a low molecular weight GTPase that is similarly C-terminally farnesylated and, when in the GTP-bound state, binds and activates Raf.[14-16] Isolated reports of *NRAS* mutations at codons 12 or 61 occur in the literature as early as 1991,[12,91] but it was the lung adenocarcinoma TSP that first detected the significance of a recurring mutation, Q61L, in a single experiment.[8] It is difficult to accurately calculate the mutation rate with so few observations, but it is likely between 1% to 2%. The *NRAS* Q61L mutation is oncogenic[12]; however, little has been done with regard to validating mutant NRAS as a therapeutic target in lung adenocarcinoma.

CTNNB1

In the APC pathway frequently inactivated in colorectal carcinoma, the APC-AXIN-GSK3β tumor suppressor complex acts to phosphorylate β-catenin and target it for ubiquitin-mediated degradation.[92,93] Mutations of the gene encoding β-catenin, *CTNNB1*, are frequent in colorectal carcinoma and endometrial carcinoma and tend to impact or even eliminate APC-dependent serine and threonine phosphorylation sites, resulting in oncogenic stabilization of β-catenin.[94,95] Recurring somatic mutations encoding *CTNNB1* G34E, S37C, and S37F were initially described in lung adenocarcinoma in 2001 and confirmed in the TSP experiment.[8,96] Although the number of observations is still too low to define the overall mutation frequency in lung adenocarcinoma, the current best estimate is between 1% to 4%. The observed mutation profile mirrors the previously described pattern of mutations in endometrial carcinoma rather than colorectal carcinomas, also characterized by a high frequency of microsatellite instability; the reasons for this remain unclear.[94]

Exploratory preclinical experiments inhibiting β-catenin signaling in colorectal carcinoma with RNA interference against *CTNNB1*, *KRAS*, and transcription factor *ITF2* or small molecules that stabilize the APC-AXIN-GSK3β destruction complex look promising,[97,98] but these approaches may not easily

translate to lung adenocarcinoma therapies for patients harboring stabilizing mutations of *CTNNB1*. Thus, no real progress has been made in targeting oncogenic mutant forms of *CTNNB1* in lung cancer.

Other Rare Activating Mutations

Rare activating mutations of additional known oncogenes have also been detected in lung cancer. Because they have been observed so infrequently, it is not clear whether these mutated genes would have any value as therapeutic targets in lung adenocarcinoma, unless the appropriate inhibitors were developed due to indications of utility in a different tumor type. For example, a mutation of the serine/threonine kinase gene *AKT1*, which acts downstream of *PIK3CA* to promote cell proliferation, motility, and viability, was identified in the lung adenocarcinoma TSP experiment.[8,99] This mutation, E17K, was previously found in 8% of breast cancer samples, 6% of colorectal cancer samples, and 5% of bladder cancer samples and was demonstrated to be activating and oncogenic.[100,101] Intriguingly, *AKT1* E17K has also been reported in 2 lung squamous cell carcinomas.[102] It is possible that the development of inhibitors for activated AKT in other tumor types could benefit the few lung adenocarcinoma patients who express the E17K substitution as well.

A somatic activating mutation of the dual-specificity kinase *MEK1*, encoding K57N, has also been reported in 2 lung adenocarcinoma samples.[103] MEK1 functions downstream of RAS and RAF proteins to activate ERK1 and ERK2. Unlike expression of wild-type *MEK1*, expression of *MEK1* K57N supports IL-3–independent proliferation of Ba/F3 cells, indicating that the somatic allele encoding K57N is oncogenic.[103] Although MEK inhibitors did not show efficacy in unselected NSCLC patients in phase II clinical trials, it possible that such inhibitors might yet be effective in the small population of lung adenocarcinoma patients who harbor activating *MEK1* mutations.[19-21]

The *PTPN11* gene, which encodes the nonreceptor tyrosine phosphatase SHP2, has pleiotropic effects in the cell. SHP2 enhances RAS-ERK signaling and, under certain circumstances, can also affect PI3K-AKT signaling and RHO activity; however, the precise mechanism of these effects is not completely understood.[104] Activating somatic mutations of *PTPN11* have been reported in several childhood hematopoietic cancers[105] and more recently in lung adenocarcinoma.[8,106-108] There is some *in vitro* evidence that a combination of MEK inhibition and inhibition of mTOR, a kinase downstream of PI3K and AKT, might be effective against tumor cells harboring activating mutations of *PTPN11*.[109]

It is difficult to judge the importance of these rare activating mutations without a better understanding of their frequency in lung adenocarcinoma patients. The TSP experiment, which included 188 samples, was insufficiently powered to reliably detect genes mutated in less than 5% of samples. Sequencing of larger numbers of samples will likely permit a more accurate estimation of the frequency of these mutations in lung cancer patients. Even at 1%, these rare mutations could occur in thousands of patients, justifying efforts in targeted therapies.

Significantly Mutated Tumor Suppressor Genes

Inactivation of tumor suppressor genes also plays a role in the development of lung adenocarcinoma. Although the design of targeted therapies for tumor suppressor genes is not as straightforward as for oncogenes, approaches such as targeting activated genes downstream of an inactivated tumor suppressor gene or identification of synthetic lethal interactions are being examined. Several tumor suppressors were significantly mutated in the TSP experiment (Fig. 1), including those already known to be involved in lung adenocarcinoma, such as *TP53*, *STK11*, and *CDKN2A*, and those not well characterized in lung adenocarcinoma, including *NF1*, *ATM*, *APC*, and *RB1*. Interestingly, many of these tumor suppressor genes lie in the same pathways as the mutated oncogenes described above.

TP53

TP53 is the most frequently mutated gene in lung adenocarcinoma, with somatic mutations found in close to 70% of patient samples (Fig. 1). Its protein product, p53, activates transcriptional programs that induce cell cycle arrest, apoptosis, or senescence in response to diverse cellular stresses.[110] Recurring mutations of *TP53* in lung adenocarcinoma were described in 1989 and include missense mutations, frameshift insertions and deletions, splice site mutations, and nonsense mutations.[8,111,112] These mutations can result in simple loss of protein function, dominant negative activity by virtue of dimerization with wild-type p53, and even neomorphic gain of function, consistent with oncoprotein activity.[113] Indicating a possible essential function, homozygous deletions of *TP53* are rare in cancer.[114,115] However, *MDM2*, an oncogene encoding an E3 ubiquitin ligase that targets p53 for degradation, is a target of focal amplification in lung adenocarcinoma.[115,116]

Several approaches have been taken to targeting mutant p53.[116,117] Gene therapy with adenovirally delivered wild-type *TP53* was approved for the treatment of head and neck cancer in China (Gendicine, Shenzhen SiBiono GeneTech, Shenzhen, China)[118] and is in phase III clinical trials for head and neck cancer in the United States (Advexin, Introgen Therapeutics, Austin, TX). Some mutations adversely affect the stability of the core domain of p53[119] yet are still expressed or even overexpressed; for this subset of mutants, compounds that stabilize the native protein conformation appear to restore its tumor suppressor activity.[120,121] Such compounds have not yet advanced beyond preclinical studies. A third intriguing approach to targeting mutant p53 involves abrogating the G2 checkpoint of the cell cycle in the presence of traditional cytotoxic chemotherapeutics that cause DNA damage; because loss of p53

activity abolishes the G1 checkpoint, treatment with G2 checkpoint inhibitors forces the tumor cells into mitosis with irreparable DNA damage.[122] Several inhibitors of the G2 checkpoint protein CHK1 are in phase I clinical trials in combination with cytotoxic agents.[122] It remains to be seen which of these approaches will result in clinical benefit, if any.

STK11

STK11 encodes the serine/threonine protein kinase also known as LKB1 (liver kinase B1), which phosphorylates and activates AMP-activated protein kinase (AMPK) under conditions of low intracellular ATP levels; activated AMPK in turn inhibits mTOR in a TSC2- and RHEB-dependent manner.[123] Truncating germline mutations of *STK11* were identified in patients with Peutz-Jeghers syndrome, a rare hereditary disease characterized by predisposition to several types of malignancies.[124] More recently, similar truncating but somatic mutations were found in lung adenocarcinoma patients.[125] In the TSP experiment, 34 patients (18%) harbored somatic mutations of *STK11*, including 10 nonsense mutations, 9 frameshift insertions or deletions, and 6 splice site mutations as well as 9 missense mutations.[8] *STK11* knockout exacerbated *KRAS* G12D tumorigenesis in a mouse model of lung cancer, and these mice were used to show that a combination of MEK, PI3K, and SRC inhibition caused tumor regression in this model.[126,127] Again, whether this approach has any clinical efficacy remains to be determined.

CDKN2A

Two tumor suppressor proteins are encoded by the human *CDKN2A* locus: p16 and $p14^{ARF}$.[128] Interestingly, these 2 transcripts utilize distinct first exons and alternative reading frames in the remaining exons. p16 functions to inhibit activity of the cyclin-dependent kinases CDK4 and CDK6, inducing arrest in G1 of the cell cycle by blocking phosphorylation of the RB protein. In contrast, p14 binds and inhibits MDM2-mediated ubiquitination and degradation of the tumor suppressor p53. Somatic mutations of *CDKN2A* were first described in lung adenocarcinoma in 1994.[129] Subsequently, many mechanisms for *CDKN2A* inactivation were uncovered in addition to nonsynonymous point mutations, including homozygous deletions, frameshift and nonsense events that result in protein truncation, and promoter methylation.[130-132] Only 9 samples in the TSP experiment (5%) were demonstrated to harbor mutant *CDKN2A*; however, the small size of this gene was a major factor contributing to statistical significance.[8] Specifically, if we imagine one driver event per gene, then the driver mutation rate per MB will be higher for small genes, which allows us to better distinguish drivers from the background (M. Lawrence *et al*., unpublished data).

Importantly, whereas *CDKN2A* and *TP53* mutations frequently coexist in lung adenocarcinoma, RB and p16 inactivation appear to be largely mutually exclusive.[8,133,134] This is consistent with the idea that release of CDK inhibition is the required effect of *CDKN2A* somatic alterations. Several CDK inhibitors are in clinical trials, but none has yet been developed into an effective therapy for lung cancer.

NF1

The most significantly mutated gene in the TSP experiment that was not previously appreciated in lung cancer is the tumor suppressor gene *NF1*. *NF1* was originally cloned as the tumor suppressor gene disrupted in the germline of patients with neurofibromatosis, characterized by benign Schwann cell tumors called neurofibromas, as well as an increased risk of malignancies.[135-138] The NF1 protein product is a GTPase activating protein for RAS, stimulating hydrolysis of bound GTP to GDP on RAS, resulting in downregulation of RAS protein activity.[136] In the TSP experiment, 7% of patients harbored somatic mutations of *NF1*, including 4 nonsense mutations, 5 splice site mutations, and 1 frameshift deletion as well as 6 missense mutations.[8] Furthermore, 3 patients harbored 2 *NF1* mutations each. Although it is not known if these mutation pairs are in cis or trans, this observation is potentially consistent with Knudsen's 2-hit hypothesis for tumor suppressor gene inactivation.[139] Because the primary effect of *NF1* inactivation appears to be upregulation of RAS pathway signaling, the therapeutic approaches under investigation for tumors harboring *KRAS* mutations described above may also be useful in patients with somatic mutations of *NF1*.

ATM

Of 188 lung adenocarcinoma patients whose DNA was sequenced in the TSP experiment, 7% were found to harbor somatic mutations of the serine/threonine protein kinase gene *ATM*.[8] These mutations included 10 missense mutations, 2 frameshift deletions, a splice site mutation, and a nonsense mutation, consistent with loss of function. Germline mutations of *ATM*, or "ataxia telangiectasia mutated," are found in patients affected with the familial disease ataxia telangiectasia (AT), characterized by pleiotropic symptoms including sensitivity to ionizing radiation and predisposition to hematopoietic malignancies.[140] ATM was subsequently shown to induce the G1 and G2 cell cycle checkpoints in response to DNA damage by phosphorylation of p53 and CHK2, respectively, although it is now known that ATM has a large number of substrates that contribute to the DNA damage response.[141,142]

Inactivating somatic mutations of *ATM* have been identified in several hematopoietic cancers, including T cell prolymphocytic leukemia, B cell chronic lymphocytic leukemia, and mantle cell lymphoma.[143] However, recurring mutations of *ATM* had not been previously recognized in solid tumors prior to the

TSP experiment. Inhibition of ATM has been shown to enhance cellular sensitivity to ionizing radiation, and there is some anecdotal evidence that the radiosensitivity of mantle cell lymphoma tumors can be in part attributed to ATM inactivation.[144-147] To my knowledge, possible radiosensitization of lung adenocarcinoma tumor cells harboring mutations of *ATM* has not yet been explored.

APC

The familial adenomatous polyposis coli gene, *APC*, was identified by positional cloning in patients displaying a characteristic hereditary predisposition to colorectal tumors.[92,148] *APC* mutations, primarily nonsense mutations and frameshift insertions and deletions encoding truncated proteins, were subsequently identified in a majority of sporadic colorectal adenomas and carcinomas.[149] Somatic *APC* mutations have furthermore been described in several other solid tumors, most prominently in gastric and pancreatic cancers.[150,151] In the lung adenocarcinoma TSP experiment, 13 somatic mutations of *APC* were detected in 11 patients, for a frequency of 6%.[8] Mutations included 3 nonsense mutations, 4 frameshift insertions and deletions, a splice site mutation, and 5 missense mutations, again consistent with loss of function of a tumor suppressor gene.

As described above, APC is a scaffolding protein that forms a tumor suppressor complex with AXIN and GSK3β that phosphorylates β-catenin and targets it for proteasomal degradation.[92] Because *APC* and *KRAS* mutations frequently co-occur in lung adenocarcinoma, the aforementioned therapeutic approach involving simultaneous inactivation of *KRAS*, *CTNNB1*, and *ITF2* may be applicable to lung adenocarcinoma patients harboring inactivating mutations of *APC*.[97] Small molecules that stabilize the APC-AXIN-GSK3β destruction complex or destabilize interaction of β-catenin with TCF/LEF transcription factor family members may likewise constitute a reasonable therapeutic approach for such lung adenocarcinoma patients.[98,152] Because of the well-characterized frequency of inactivating *APC* mutations in colorectal carcinoma, these approaches are primarily being explored in the context of colon cancer; it remains to be seen whether any evidence will be produced supporting these approaches in lung adenocarcinoma cells harboring mutations of *APC*.

RB1

Although alterations of *RB1* in small cell lung cancer have been known for many years,[153,154] recurring and statistically significant *RB1* mutations were only recently found in lung adenocarcinoma.[8] Mutations of the tumor suppressor gene *RB1* were identified in only 7 patients in the TSP experiment, for a frequency of 4%; however, only nonsense mutations, frameshift deletions, and splice site mutations were observed, all of which would be expected to result in a truncated protein product and occur infrequently, thus increasing statistical significance.[8]

RB associates with and modulates activity of the E2F family of transcription factors. In the classic paradigm, RB binds and sequesters E2Fs, thus inhibiting transcription of E2F target genes involved in cell cycle progression and growth promotion; phosphorylation by activated CDKs relieves this repression and permits transcription of the E2F target genes.[155] Layers of complexity of RB function have since been uncovered.[156] $Rb1^{+/-}$ mice develop pituitary and thyroid tumors; E2F-1 and E2F-4 losses ameliorate tumorigenesis in this model, whereas *Skp2* loss causes overt synthetic lethality of aberrant pituitary melanotroph cells.[157-159] However, there are currently no data to support these or any other putative therapeutic approaches for lung adenocarcinoma cells harboring *RB1* mutations.

Next-Generation Sequencing Data

Much has thus been learned from exon-directed Sanger sequencing of lung adenocarcinoma samples. Several issues affect these experiments, however, including loss of power to detect tumor-specific somatic mutations in the presence of stromal contamination and limiting mutation detection to a subset of coding sequences. The falling cost of sequencing has recently enabled whole-exome and whole-genome cancer sequencing projects, which provide mutation analysis in a completely unbiased manner. Data collection for next-generation sequencing methods is moreover "digital," collected for individual molecules, such that power to detect low-abundance alleles, whether because of stromal contamination or tumor heterogeneity, is dependent only on the depth of coverage. The whole-genome sequence from a single lung adenocarcinoma patient has already been reported.[160] As whole-exome and whole-genome data from large numbers of samples are generated, a more global snapshot of somatic mutation in lung adenocarcinoma will emerge, providing a comprehensive view of the lung adenocarcinoma genome and putative therapeutic targets.

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