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CANCER DRIVER MUTATIONS IN PROTEIN KINASE GENES

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Abstract

Recent studies investigating the genetic determinants of cancer suggest that some of the genetic alterations contributing to tumorigenesis may be inherited, but the vast majority are somatically acquired during the transition of a normal cell to a cancer cell. A systematic understanding of the genetic and molecular determinants of cancers has already begun to have a transformative effect on the study and treatment of cancer, particularly through the identification of a range of genetic alterations in protein kinase genes, which are highly associated with the disease. Since kinases are prominent therapeutic targets for intervention within the cancer cell, studying the impact that genomic alterations within them have on cancer initiation, progression, and treatment is both logical and timely. In fact, recent sequencing and resequencing (i.e., polymorphism identification) efforts have catalyzed the quest for protein kinase ‘driver’ mutations (i.e., those genetic alterations which contribute to the transformation of a normal cell to a proliferating cancerous cell) in distinction to kinase ‘passenger’ mutations which reflect mutations that merely build up in course of normal and unchecked (i.e., cancerous) somatic cell replication and proliferation. In this review, we discuss the recent progress in the discovery and functional characterization of protein kinase cancer driver mutations and the implications of this progress for understanding tumorigenesis as well as the design of ‘personalized’ cancer therapeutics that target an individual’s unique mutational profile.

1. Introduction

1.1. Modern Cancer Genomics Initiatives

The Human Genome Project (HGP) and other DNA sequencing initiatives have provided a number of important insights into the diversity of life, both within and across different species, as well as insight into the overall structure and organization of genes and gene regulatory elements [1,2]. In addition, the development of high-throughput molecular genetic technologies such as mRNA and protein microarrays have provided tools for surveying the tissue and temporal patterns associated with gene expression. Furthermore, since the publication of the draft haploid human genome sequence, a number of efforts in genetics research have focused on the identification and cataloguing of naturally occurring DNA

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sequence variations. For example, large-scale resequencing and polymorphism characterization efforts such as those sponsored by the International Haplotype Map Initiative (HapMap) and the SNP Consortium (TSC) have focused on the characterization of the patterns of common variations within the human genome [3]. The Human Cancer Genome Project (HCGP), was initially proposed a few years ago as a 10-year, \$1.5 billion mega-effort to derive a complete catalog of inherited and acquired genomic variations that occur in tumors and to characterize the functional consequences of these genomic alterations. Despite the original criticism of this colossal project within the cancer community [4], a 3 year, \$100 million pilot project, cleverly titled The Cancer Genome Atlas or 'TCGA' has been launched and, according to the very recent report, the initial results are encouraging and point to potential new therapies [5,6] (<http://cancergenome.nih.gov/>).

As important as these initiatives are, their initial focus is on the mere identification and cataloguing of variations, and not necessarily on understanding the biological significance of the identified variations. A central goal of cancer research involves the discovery and functional characterization of the mutated genes that drive tumorigenesis [7,8] as opposed to those merely accumulating as a result of tumorigenesis. Recent developments in differentiating 'driver' from 'passenger' mutations have underscored the importance of integrating genetic, functional, and structural insights in unveiling just how specific genetic alterations driving tumorigenesis may lead to overt and distinct clinical phenotypes that require unique preventative and treatment strategies.

1.2. Protein Kinases Genes, Tumorigenesis, and Cancer Treatment

The first consistent genetic abnormality associated with human cancer was detailed in the publication of the 1960 discovery of the Philadelphia chromosome, a fusion of two protein kinases, breakpoint cluster region (BCR) and Abelson leukemia virus tyrosine kinase (ABL), in chronic myelogenous leukemia (CML) [9]. However, since no consistent chromosomal alterations were found in other forms of cancer, it was thought that chromosome alterations were probably a result of cancer, not a cause. In 1976, John Bishop and Harold Varmus demonstrated that oncogenes, i.e., genes which participate in the onset of cancer (in particular the tyrosine kinase Schmidt-Ruppin A-2 viral oncogene homolog (SRC)), were defective proto-oncogenes native to a normal cell [10], solidifying the role of genetic alterations in initiating and mediating tumorigenesis rather than merely arising as a result of tumorigenesis. Thus, SRC became the first human proto-oncogene to be identified, and a flood of other protein kinase proto-oncogenes were discovered soon after.

By identifying certain genomic events as driving or contributing to tumorigenesis in this fashion, treatments have been designed to specifically ablate their tumorigenic effects. In 1998, the drug Herceptin became the first protein kinase inhibitor to be approved for treating cancer [11]. In fact, many genetic changes have been found to be strongly associated with clinical response to inhibitors targeting protein kinase lesions. Most notably, the dependence of chronic myeloid leukemia (CML) on the translocated BCR-ABL kinase led to the discovery of dramatic CML responses to small-molecule inhibitors targeting BCR-ABL [12–16]. However, it was not until 2001 when the FDA approved Gleevec, a tyrosine kinase inhibitor with activity against the gene BCR-ABL [17–20], that the true potential of kinase-targeted therapies was realized. In fact, much of the success of Gleevec was due to its efficacy only in the treatment of cancers driven by Philadelphia chromosome, solidifying the connection between mutated kinases driving tumorigenesis and the successful treatment of cancers by targeting those mutant. Since the extraordinary success of Gleevec, protein kinases have entered the spotlight as premier oncology targets [21–23].

Protein kinases are an evolutionarily-related family of more than 500 proteins encoded in the human genome, that serve as signaling switches sharing a unique catalytic core [24–30]. This

conserved core catalyzes the transfer of the γ -phosphate of ATP to the hydroxyl group of serine, threonine, and tyrosine residues in protein substrates. Although the conserved residues of this catalytic domain were recognized by sequence alignments, a landmark for understanding the molecular basis for kinase function was elucidation of the crystal structure of cAMP-dependent protein kinase, protein kinase A (PKA) in 1991 by Dr. Susan Taylor's laboratory [31–33]. Since this pioneering study, the crystal structures of nearly 70 different protein kinases have been determined [34–36]. Protein kinase crystal structures have revealed considerable structural differences between closely related active and highly specific inactive forms of the enzyme. Of all protein families known to contribute to tumorigenesis, protein kinases are the most overrepresented family [37,38]. Protein kinases act as both tumor suppressors and proto-oncogenes in normal, healthy cells; thus, mutations in protein kinases tend to promote a wide variety of tumorigenic activities. Kinase mutations may activate proliferative pathways, causing genomic instability by abrogating cell cycle checkpoints, inhibit or abolish DNA damage response, deactivate apoptotic pathways, and/or promote angiogenesis and cell motility. Since protein kinases play a central role in DNA damage response as well as cell cycle checkpoints, numerous loss of function mutations in kinases have been associated with inhibition of apoptosis or acquisition of a 'mutator' phenotype and eventual tumorigenesis.

1.3. Example Kinase Drivers

Mutations in ataxia telangiectasia mutated (ATM), known to play a role in cell cycle checkpoints and DNA damage response, are involved in hereditary ataxia-telangiectasia [39] and result in an elevated risk for mature T-cell leukemia. Various studies have demonstrated an association of ATM gene with blood cancers [40], as well as breast cancer [41]. DNA damage-response genes such as ataxia telangiectasia and Rad3 related (ATR) and other checkpoint kinases, such as checkpoint homolog 1 (CHK1), have been associated with cancer, including sporadic stomach tumors [42]. CHK1 mutations may inhibit apoptosis and/or cause microsatellite instability. Other examples include an association of DNA-activated protein kinase (DNAPK) mutations with mismatch repair deficient tumors [43], and checkpoint homolog 2 (CHK2) mutations in a wide range of cancers, including breast, colon, kidney, prostate, and thyroid cancers [44]. In addition to point mutations, there are numerous examples of the involvement of protein kinases in cancer through over-expression, translocation, deletion, and amplification. However, the focus of this review will be on point mutations that drive tumorigenesis. Since many of the specific activating point mutations occur in receptor tyrosine kinases, these kinases have inevitably been studied in much greater detail than others, however the lessons learned are likely to apply to analysis of non-receptor tyrosine kinases as well as other protein kinase families.

2. Cancer-Related Somatic Mutations in Protein Kinases

2.1. Hunting for Cancer Mutations through Genomic Sequence Comparisons

Recent exon resequencing studies of gene families involved in cellular signaling pathways, such as tyrosine kinases, tyrosine phosphatases, and phosphatidylinositol 3-kinases have identified many potential tumorigenic driver mutations [45–55]. High-throughput screens of the tyrosine kinome and tyrosine phosphatome have been performed in a spectrum of human malignancies, including colorectal cancer [46–48], lung cancer [49–53], breast cancer [54], human testicular germ-cell tumors [55] and glioblastoma multiforme [56]. In particular, a significant role of murine sarcoma viral oncogene homolog B1 (BRAF) mutations in melanoma was uncovered through genomics-based sequencing [45]. In non-small cell lung carcinoma, systematic resequencing of tyrosine kinase genes identified somatic mutations within the epidermal growth factor receptor (EGFR) tyrosine kinase gene [49–53].

Targeted resequencing of the kinome in cancer has suggested that protein kinase cancer drivers are dispersed across the entire family. In a recent study, resequencing of 518 protein kinases in 26 primary lung neoplasms and 7 lung cancer cell lines revealed 188 somatic mutations distributed across 141 kinase genes [53]. Of these 188 somatic mutations, three fourths were likely to be passengers, or, rather, incidental mutations of little or no functional consequence that arise simply as the result of the random mutagenic processes underlying the development of cancer. No single kinase was commonly mutated, suggesting that several infrequently mutated kinases contribute to tumorigenesis.

A complete sequencing of kinase exons was recently completed that focused exclusively on breast cancer [54]. This study examined the coding sequence of 518 protein kinases in 25 breast cancers and identified 90 somatic mutations, with a marginal excess of missense mutations and no clustering of mutations in any single kinase gene, kinase family, or functional domain. In some cases, no point mutations were observed, as was the case in the kinome resequencing study of testicular cancer [55]. Another recent sequencing study described a systematic analysis of 13,023 well-annotated human protein-coding genes in 11 breast and 11 colorectal cancers in an initial 'discovery' screen, followed by an analysis of 24 additional breast or colorectal tumors in a 'validation' screen [57]. This study identified 189 genes displaying somatic mutations (average of 11 per tumor) that were mutated at significant frequency. Several plausible candidate driver mutations have been identified from this data in the conserved, functional kinase domains, including the glycine-rich P-loop and the activation segment. These kinase subdomains frequently harbor oncogenic mutations in known cancer genes such as EGFR, FMS-related tyrosine kinase 3 (FLT3), Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) and BRAF.

In a more comprehensive study, human cancers, including breast, lung, colorectal, gastric, testis, ovarian, renal, melanoma, glioma and acute lymphoblastic leukemia, were screened for somatic mutations in the exons and splice junctions of the 518 protein kinase genes, amounting to a total sequencing effort involving 274 Mb of cancer genome [59]. Of the 210 analyzed cancers of varying types, over a 1,000 somatic mutations were observed, of which approximately 158 were expected to be drivers, suggesting that most somatic mutations in cancer cells are likely to be passenger mutations. Furthermore, this study also detected biases towards particular kinase functional domains and inferred that approximately 120 of the 518 screened genes may harbor a driver mutation and therefore be designated as 'cancer causing' genes – a much larger number than was originally anticipated. [59]. These 158 drivers were confined to 66 of the 210 cancer samples. Thus, while protein kinases have a clear role in tumorigenesis, commonly mutated protein kinases in cancer appear to be the exception to the rule. An extreme example of this exception involves the recurrent BRAF kinase mutations in melanoma, where approximately 60% of melanomas carry a specific mutation within the catalytic core [45]. Hence, with the exception of this BRAF mutation in melanoma, the vast majority of kinase driver mutations are expected to be distributed across a large set of protein kinases.

In a tumor resequencing study by Wood and colleagues [61] – the most comprehensive tumor resequencing study performed to date - the authors described the mutational spectrum of 18,191 distinct genes in 11 breast and 11 colorectal tumors through a systematic sequencing of exons, finding at least one nonsynonymous mutation in 1718 genes out of the 18,191 analyzed. Of these genes, 280 were considered to be candidate cancer genes, and out of those, 40 genes were analyzed in a separate cohort of 96 patients. Most of those genes were found to be mutated in less than 5% of the tumors studied, suggesting that the genetic landscape of breast and colorectal cancers includes few commonly mutated genes along side over 200 genes mutated at a lower frequency. The discovery that a large number of cancer driver mutations may not

occur frequently is exemplified in other sequencing studies of the kinome, where specific kinases are sometimes mutated in a small fraction of tumors of a given type [59].

2.2. Differentiating Drivers from Passengers

To distinguish genes likely to drive tumorigenesis from passenger mutations, a few early studies took a purely statistical approach [57,59]. These statistical analyses rely upon indications of the positive selection of mutants during cell proliferation; i.e., these methods try to identify statistical biases in the distribution of mutations across different categories of mutations that might reflect a tumorigenic proliferative advantage. These categories can include the mutation type, i.e. missense, nonsense, splice site, etc., or the distribution across groups of proteins (as defined by phylogeny), or even the distribution of mutations across particular functional domains or functional sites. Positive selection methods rely upon the determination of a baseline mutation rate and the expected distribution of mutations as a result of this rate. Similar statistical methods have been applied in attempts to identify individual cancer drivers [61]. However, such approaches have generated controversy and have MET with fierce criticism, culminating in scientifically heated exchanges involving several leading cancer sequencing labs [62–64]. For example, it is thought that such statistical methods are highly sensitive to the determination of a background mutation rate, and statistical power based on sample size. Thus, it has been argued that after correcting the statistical analysis and using a background mutation rate that better fits the data, the statistical evidence for almost all of the yet unknown candidate cancer drivers is reduced dramatically [64]. Thus, purely statistical approaches to evaluate the impact of DNA sequence variants on tumorigenesis have demonstrated the difficulties in distinguishing driver from passenger mutations [57–64]. Despite this, there is the belief that given that certain genes appear to be consistently amplified in their expression and/or number within and across different tumors, certain genes must be playing a role in tumorigenesis that is driven by some perturbation. Thus, the ‘oncogene addiction’ hypothesis suggests that despite the accrual of numerous genetic alterations over the maturation of a tumor, cancer cells appear to remain reliant upon particular oncogenic pathways and may become addicted to the continued activity of specific activated oncogenes [65–67].

2.3. Sequencing and Functional Profiling of Kinase Driver Mutations

To obtain insight into the specificity and sensitivity of sequencing screens in identifying functional ‘driver’ genomic alterations, a fascinating study by Loriaux and colleagues exploited high-throughput DNA sequence analysis and functional assessment of candidate variations to screen exons encoding the activation loop and juxtamembrane domains of 85 tyrosine kinase genes in 188 acute myeloid leukemia (AML) patients without FLT3 or c-KIT mutations [68]. The screen identified 30 not previously reported nonsynonymous sequence variations in 22 different kinases. Remarkably, resequencing and subsequent functional analysis of the observed mutants for constitutive tyrosine kinase activation have collectively demonstrated that a significant proportion of newly identified nonsynonymous sequence variants may not have functional significance. Of 30 nonsynonymous tyrosine kinase mutants found in the 188 AML samples, only one showed constitutive phosphorylation, and none transformed Ba/F3 cells to factor-independent growth [68]. This functional analysis further emphasizes the fact that the majority of nonsynonymous sequence variations may be functionless passengers rather than driver mutations, thereby underscoring the importance of functional assessment of any putative genomic alteration thought to be a driver. The authors of this study have concluded that functional analysis of a large number of mutations identified in sequencing screens may be necessary to determine which mutations are drivers and hence likely therapeutic targets. A high-throughput mutational screen of the tyrosine kinome in chronic myelomonocytic leukemia (CMML) has shown that point mutations in conserved hot spots within the activation loop in leukemia-associated tyrosine kinases, are rare in patients with CMML [69].

Interestingly, no novel activating mutations in tyrosine kinases have been discovered in large cohorts of patients with AML [69], which is consistent with a low number of discovered somatic mutations in earlier high-throughput resequencing studies [68]. In another study by Tomasson and colleagues, the kinase domains of 26 tyrosine kinases, selected on the basis that they were expressed in *novo* acute myeloid leukemia (AML) tumors, were resequenced [70]. Only 4 novel somatic mutations, occurring at conserved residues, were identified in the Janus kinase 1 (JAK1), death domain receptor family member 1 (DDR1), and neurotrophic tyrosine kinase receptor type 1 (NTRK1) genes. Interestingly, the authors suggested that mutations in one of these newly discovered genes may preclude the need for mutations in additional family members. In contrast, activating mutations in highly expressed and commonly mutated FLT3, KIT and Janus kinase 2 (JAK2) genes may be additive [70]. An impressive high-throughput platform has been recently used to interrogate the entire FLT3 coding sequence in AML patients without known FLT3 mutations and experimentally test the functional consequences of each candidate tumorigenic genomic alteration [71]. This approach successfully identified 9 candidate leukemogenic alleles, of which 4 are gain-of-function mutations that result in constitutive kinase activation. The important insights of this pioneering study have shown that 1. rare driver variants may occur at frequencies indistinguishable from passenger mutations; 2. somatic mutations in the kinase activation loop, which was typically thought to exclusively harbor driver mutations, may not be associated with a detectable gain of function; and 3. statistical models can be useful in identification of candidate cancer genes, but do not directly predict the ability of individual alleles to contribute to transformation activation. In another interesting study [72], it was discovered that somatic doublet mutations are present at high frequency in the epidermal growth factor receptor (EGFR) tyrosine kinase domain in lung cancers. About half of all doublets contain one or two of 12 distinct missense mutations at five amino acids: E709, G719, S768, T790 and L861. The data imply that most EGFR doublets are not consistent with the driver vs. passenger mutation mechanism, but rather that EGFR doublets may have been functionally selected from two individually suboptimal mutations that, in combination, may have enhanced oncogenic potential. Therefore, resequencing studies that employ purely statistical models or *in silico* approaches to identify driver mutations and infer their biological significance must be complemented by functional assays.

2.4. Integrated Computational Approaches to Driver Prediction

Machine learning methods, designed initially to differentiate between inherited disease causing polymorphisms and neutral variations, have shown some promise in identifying and differentiating cancer driver mutations from neutral passenger mutations. It has been observed that known cancer drivers display characteristics similar to Mendelian disease-associated mutations rather than complex disease-associated variations [73]. Therefore, it is likely that cancer driver mutations are sufficient for promoting a tumorigenic mechanism and, further, this observation may explain the mutual exclusivity of particular driver mutations in cancer. For example, it has been observed that FLT3 and KIT activation appear to be mutually exclusive in AML, and that BRAF and neuroblastoma RAS viral oncogene homolog (NRAS) mutations appear to be mutually exclusive in melanoma [74]. Given the Mendelian character of cancer driver mutations, a prediction method, known as 'CanPredict,' was developed to distinguish driver from passenger mutations [75]. This method leverages sequence conservation based on the 'SIFT' score [76], deviations from a hidden markov model score for protein domain identification, and gene ontology information. Though quite powerful, generalized prediction methods such as CanPredict may fail to achieve the sensitivity and specificity attainable by prediction models tailored to individual protein families. An alternative method, focused upon protein kinase predictions, was developed by the authors of this review [77–79]. The two methods have complimentary strengths and weaknesses. The CanPredict method can be applied to the whole genome, but is restricted to making predictions on mutations falling within functional domains. On the other hand, the kinase specific method

[77] is capable of making predictions outside of functional domains, but is restricted to the protein kinase gene family. By leveraging structural, phylogenetic, and physiochemical attributes of kinases, a support-vector machine (SVM) analysis model predicted known cancer driver mutations in protein kinases contributing to cancer progression. Using multiple subdomain based alignments, this scheme was used to identify a number of structurally conserved positions within the protein kinase catalytic core that appear to be frequent targets of tumorigenic mutations [78]. We have also analyzed the structural location of common and disease-associated SNPs in the catalytic domain of protein kinases and found that common SNPs are randomly distributed within the catalytic core and known disease SNPs consistently map to regulatory and substrate binding regions [79]. Application of this method to kinome resequencing data largely agreed with previous estimates of the number of drivers observed in various sequencing efforts; i.e., that a small number of cancer driver mutations were predicted to be dispersed over a group of infrequently mutated protein kinases.

3. Cancer Mutation Hotspots in Protein Kinases

Although the kinase catalytic domain is highly conserved, protein kinase crystal structures have revealed considerable structural differences between the closely related active and highly specific inactive forms of kinases [34–36]. The structures adopted by inactive kinases generally differ dramatically in the vicinity of the activation loop residues, in contrast to the well-conserved structures seen in active kinases [80–88]. These distinct inactive conformations are a reflection of different regulatory mechanisms that have evolved to allow individual protein kinases to react to their specific set of regulatory signals. Thus, protein kinases interconvert between functionally important active and inactive states of the enzyme, and the phosphorylation of key residues can shift the balance between these states [34–36]. Evolutionary functional conservation and conformational plasticity of the kinase catalytic domain allows kinases to effectively achieve a dynamic equilibrium between active and inactive forms. This equilibrium ultimately facilitates regulation of their catalytic activity and recognition by other molecules. For example, the conformational landscape of ABL kinase, which can include active, inactive, intermediate, and inactive-like conformations, has confirmed that diverse structures of the kinase activation loop may reflect natural kinase conformations [84–88]. Therefore, it is probable that activating somatic mutations disrupt this equilibrium and favor the active conformation, either by destabilizing the inactive state or stabilizing the active state of the protein kinase and this mechanism may be a play in certain mutations' ability to 'drive' tumorigenesis.

Although the majority of protein kinase cancer drivers are likely to be rare, there are examples of notable cancer driver hotspots that may shed some insight on the evolutionary and structural mechanisms underlying activation of proto-oncogenes [78]. Given that activating mutations must result in very specific changes in order to activate a protein kinase, and the abundance of receptor tyrosine kinases in growth factor or proliferative roles, much of the indepth mutational information is focused on receptor tyrosine kinases. In particular, existing experimental data has shown that structurally conserved mutants M918T in multiple endocrine neoplasia and medullary thyroid carcinoma 1 oncogene (RET) [89–92] and M1250T in hepatocyte growth factor receptor (MET) kinases [93–98] are associated with oncogenic activation and display the highest transforming potential, leading to uncontrolled cell proliferation and tumorigenesis. Moreover, this mutational hotspot is also shared in activin A receptor type II-like 1 (ALK1) (M-R), MET (M-T), RET (M-T), transforming growth factor-beta receptor type II (TGFbR2) (M-V). Three of the four mutations are transitions from methionine to either arginine or threonine, indicating that these mutations may disrupt the hydrophobic binding pocket by introducing polar amino acids. M918T in RET and M1250T in MET are situated within the P +1 loop, which is a small motif, immediately C-terminal of the A-loop playing an important role in recognizing the residues flanking the target tyrosine in the substrate. Another prominent

cancer mutation hotspot is located four residues C- terminal to glycine of the DFG motif, in the middle of the A-loop and is shared by D1228 in MET [99–101] , D835 in FLT3 [102, 103] , D816 in KIT [104,105] as well as V600 BRAF [45,74].

It is worth noting that there was a certain confusion recently in the literature concerning the numbering of the amino acids in BRAF, and though it was originally reported that the valine at position 599 is the most commonly mutated amino acid in human cancer, it was eventually reconciled that this valine is located at position 600 [106]. A significant number of known activating mutations in this position include D1228H/N/V in MET D835E/F/H/N/V/Y in FLT3, D816E/F/V/H/I/N/V/Y in KIT and V600D/E/G/K/L/M/R in BRAF. Unlike the M918 RET and M1250 MET hotspot, which is implicated in substrate recognition, the conserved A-loop residue arguably play an important role in kinase autoinhibition and stabilization of the inactive state of the enzyme. The crystal structure of BRAF V600E revealed that activation loop residues form a hydrophobic interaction with P-Loop residues in the inactive state. Mutations in the activation loop, or the P-loop, disrupt these interactions and destabilize the inactive conformation [83]. The crystal structures of inactive, autoinhibited wild-type MET [107,108], FLT3 [109] and KIT kinases [110] have also provided insights into the molecular mechanisms underlying activation by somatic mutations in protein kinases. Given the diversity of inactive states observed across the kinome, and the dispersion of cancer driver mutations across the kinome, it is likely that observed cancer driver hotspots are exceptional cases, and that cancer drivers are generally spread out across a vast space, both phylogenetically and structurally.

4. Connecting Structural Biology and Mutant Structural Pathology

A prominent example of the importance of the characterizing the structural properties of genetic variations identified in tumors is the recent discovery and functional and biophysical characterization of somatic mutations within the epidermal growth factor receptor (EGFR) tyrosine kinase gene for non-small cell lung carcinoma (NSCLC) [49–53]. A spectrum of discovered EGFR cancer mutations were shown to induce oncogenic transformation by leading to constitutive kinase activity of EGFR. Furthermore, activating mutations in the EGFR kinase domain were shown to confer differential sensitivity to known cancer drugs, such as Erlotinib, Lapatinib, Gefitinib [81,82,111–114]. Crystal structures of the EGFR kinase domain, which have been determined previously in a free form and in the complex with Erlotinib [81], have revealed an active conformation. However, structurally different inactive conformational states of the EGFR kinase have been found in the complex with the drug Lapatinib [82] and in the complex with adenosine 5'-(β,γ -imido)triphosphate (AMP-PNP) [115]. The discovered associations between EGFR mutations and drug sensitivity have indicated that genetic alterations in specific kinases and corresponding changes in structural and interaction profiles of kinases (and not simply kinase gene expression or protein levels), can render tumors sensitive to selective inhibitors. These studies have also demonstrated that the structural perturbations of the kinases in response to activating mutations may be the ultimate cause of observed differences in drug binding [111–114].

Insights relating drug efficacy to mutation-induced protein structural perturbations observed in cancer, solidify the role of some mutations as possible ‘drivers.’ In fact, structural determination of the EGFR wild-type kinase and the L858R and G719S mutants in complexes with a range of inhibitors have recently produced molecular insights into the functional effects of protein flexibility and relevant genetic modifications of this flexibility in a way that impacts tumorigenesis and tumor maintenance [116]. A structural comparison of the EGFR L858R activation loop with the previously determined structure of the wild-type EGFR in an inactive conformation [82,115] has shown that cancer mutations can destabilize the inactive conformation and result in a kinase that is 50-fold more active than the wild-type form [116].

Hence, these studies have confirmed that cancer driver mutations may work through a mechanism of partial destabilization of the inactive conformation, ultimately causing a detrimental imbalance in the dynamic equilibrium shifted towards the active kinase conformation. Crystallographic studies of EGFR cancer driver [116–118] and ABL cancer driver mutants [119–121] have also suggested that activation by EGFR T790M and ABL T315I mutations may be triggered by conserved interactions between Phe of the conserved DFG motif and the mutated gatekeeper residue, which may unlock the inactive kinase conformation and facilitate transition to the active form.

The recent comprehensive review by Kumar and coworkers [118] has emphasized many important connections between structural and clinical studies of EGFR in human cancer, arguing that there is strong evidence for regarding EGFR cancer mutations as unique therapeutic targets. Thus, recent structural studies have not only facilitated our understanding of the functional consequences of specific cancer driver mutations in protein kinases, but have also exposed synergies between large-scale resequencing studies of kinase coding regions in tumors and targeted, disease-oriented crystallography that could lead to a powerful alternative to the current drug discovery paradigms.

5. From Mutation-Induced Structural Pathology to Personalized Medicine

The functional characterization of protein structural deformation induced by specific mutations in ABL and EGFR suggests the possibility of developing selective inhibitors that act against these specific mutations. Though understanding the precise role of genetic alterations in tumorigenesis will be challenging, the development of therapeutics designed to target unique structural pathologies induced by specific mutations is entirely consistent the concept of ‘personalized medicine’. In a review of cancer therapies that target specific tumor genomic profiles [122], the authors point out that many very specific coding variations have been implicated in mediating initial drug activity or, if identified after initiation of tumorigenesis and treatment, mediate resistance to the drug. Although the value of protein structural information in the design and development compounds used to treat conditions, such as cancer, for which specific mutations are likely to be the cause has been known for years [123,124], structure-based drug design has rarely exploited such information. Importantly, many of the existing cancer therapeutic agents, such as in CML (Gleevec) and some lung cancers (Iressa and Tarceva), are known to work better when specific genomic profiles are present in patients [122]. Remarkably, these studies suggest that prominent cancer drugs may have emerged serendipitously rather than by rational design since the relationship between the presence of mutations in the target proteins and drug efficacy was not known prior to the development and distribution of the drug. Consequently, structurally informed studies of primary and acquired drug resistance in protein kinases, based on the existence of driver mutations within those kinases, may facilitate the selection of therapy for cancer patients treated with these drugs. Recent structural and functional studies of differential inhibitor sensitivity with the wild-type and oncogenic mutants of MET and RET kinases have highlighted the upcoming challenges and pitfalls in developing such personalized cancer therapies.

According to the KinMutbase [38], there are more than 35 unique missense MET mutations and 127 missense RET kinase mutations. It has been well established that molecular mechanisms of RET [89–92] and MET [93–98] kinase oncogenic activation are largely associated with the transforming ability of specific point mutations. In this context, it has been recently reported that specific MET kinase inhibitors, including SU11274 can differentially affect kinase activity and subsequent signaling of various mutant forms of MET [95]. However, in the absence of the crystal structures of mutant forms of MET bound to SU11274 inhibitor, it was not obvious how such mutations induce resistance to specific inhibitors. Recently, a novel series of MET small molecule inhibitors that are active against wild-type and mutated

MET variants have been unveiled [125,126]. X-ray crystallography has revealed that this class of inhibitor binds MET very differently than more specific MET inhibitors which exhibit primary resistance by some MET mutants [125–127]. Although the different binding modes of these inhibitors to MET may account for their unique pattern of activity towards MET mutants, comprehensive structural studies of these inhibitors bound to MET and different MET mutants are required to fully understand these differences. A significant number of point mutations in the human RET tyrosine kinase domain can result in inappropriate kinase activation, making RET kinase a unique therapeutic target for treatment of its associated cancers. Potent inhibitors of wild-type RET and oncogenic forms of the kinase have been recently described [93]. This study described structural and biochemical analyses of the human wild type RET kinase in complexes with potent inhibitors - pyrazolopyrimidines and the 4-anilinoquinazoline ZD6474 (Zactima). These structures have provided an initial rationale as to why RET mutations at the gate-keeper residue V604 to a bulkier leucine or methionine are resistant to inhibition. However, the recent discovery that Sorafenib (BAY 43-9006) can act, not only as a highly potent inhibitor of both BRAF and RET kinases, but also as an effective inhibitor of oncogenic forms of RET kinase containing gatekeeper mutations [128] can not be rationalized based on existing crystal structures of the wild-type enzyme.

6. Future and Benefits of Whole Genome Profiling of Cancer

Both broad whole genome resequencing studies, as well as focused kinase resequencing studies, have revealed a vast array of mutations involved in tumorigenesis. An initial benefit of large scale sequencing studies will be the ability to delineate cancer subpopulations based upon mutations in more commonly mutated genes. These therapeutic improvements, exemplified by targeting leukemia patients based on BCR-ABL status, or lung cancer patients based upon EGFR mutation status, are certainly worthy of the investment in large scale sequencing studies. This knowledge, as well as the development of pharmaceuticals targeted towards these subpopulations, should provide a significant improvement in cancer therapeutic strategies and patient health, especially with the development of strategies to overcome drug resistance. Both computational and experimental diagnostic tools should be developed to facilitate the application of personalized therapeutic strategies by clinicians. However, because of the diversity of mutations observed and functionally involved in tumorigenesis, driver mutations outside of the core tumorigenic pathways will be more difficult to identify via purely statistical approaches. Sophisticated computational tools supplemented with experimental validation will need to be applied to tease out the small number of cancer drivers from the overwhelming majority of passenger mutations. While the structural diversity of wild-type protein kinases has been illuminated in recent years given the rapidly increasing body of crystal structures, structural knowledge of functionally important kinase mutants and their role in driving tumorigenesis is still very limited. More systematic structural studies of mutants are required in order to explain newly emerging data on cancer-causing mutations and differential drug sensitivity. Computational studies have begun to investigate the molecular basis of protein kinase function and the structural effects of activating mutations, which may ultimately control the activity signature of cancer drugs and determine the scope of drug resistance mutations [129–132]. Yet, computational prediction of cancer driver mutations, leveraging structural information, can be improved by a greater depth of mutant structural information. Ultimately, by integrating structural and functional knowledge of cancer mutation effects, computational approaches will inform and facilitate experiments exploring the molecular pathology of tumorigenesis and implications in rationale drug design of specific cancer therapies.

Concluding Remarks

Identifying mutations that drive tumorigenesis has important and far-reaching consequences for cancer research. Not only can the identification of such mutations shed enormous light on

genes and genomic elements contributing to cancer formation, progression, and maintenance, but can also – with the help of relevant functional and structural studies – provide very specific therapeutic targets. As made clear in this brief review, studies that have focused on cancer driver mutations in protein kinases exemplify the many problems faced in such efforts. However, merely cataloguing genetic variations in tumors – as many contemporary DNA sequencing studies do – does not provide insight into the physiological, epidemiologic, or evolutionary significance of such variations. Thus, it is now appropriate to consider the significance of those variations in mediating disease susceptibility and cancer pathogenesis.

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Table 1

A List of Recent Studies Attempting to Identify Mutations that Drive Tumorigenesis.

Study	Gene(s) Studied	Cancer(s) Studied	Methodology	Main Result(s)
Bignell et al. (2006) [55]	Kinases	Testicular	Frequency analysis	Identified a few Somatic Variants
Sjoblom et al. (2006) [56]	Kinases	Breast and Colorectal	Frequency analysis	Estimated Driver Frequencies
Thomas et al. (2007) [58]	Oncogenes	Various	Frequency analysis	Oncogene Frequencies Assessed
Greenman et al. (2007) [59]	Kinases	Various	Frequency analysis	Estimated Driver Frequencies
Kaminker et al. (2007)	Many	(General Method)	Machine learning	Algorithm for Identifying Drivers
Wood et al. (2007) [61]	Many	Breast and Colorectal	Frequency analysis	Estimated Oncogene Frequencies
Froehlin et al. (2007) [71]	FLT3	AML	Functional analysis	Single Gene Driver Frequencies
Torkamani and Schork (2008) [78]	Kinases	(General Method)	Machine learning	Algorithm for Identifying Drivers
Loriaux et al. (2008) [68]	Tyrosine Kinases	AML	Functional analysis	Identified Functional Mutations
Tyner et al. (2008) [69]	Tyrosine Kinases	CMMML	Functional analysis	Identified Functional Mutations
Tomasson et al. (2008) [70]	Tyrosine Kinases	AML	Functional analysis	Characterized Mutual Exclusivity
Chen et al. (2008) [72]	EGFR	Lung	Frequency analysis	Characterized Somatic 'Doublets'