Classification génomique des cancers du poumon: Vers un traitement personnalisé?

Genomic classification of lung cancer: Toward a personalized treatment

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RÉSUMÉ

Le cancer du poumon représente la première cause de décès par cancer dans le monde. Son incidence a augmenté en Tunisie de 17.6 cas/100.000 habitants en 1997 à 27.6 cas/100.000 habitants en 2003. Son pronostic est en train de s'améliorer depuis la découverte de nouvelles thérapies ciblées. La première d'entre elles est représentée par l'EGFR (Epidermal growth factor receptor) qui marque cette année (2014), son 10ème anniversaire. D'autres cibles thérapeutiques ont été identifiées et sont représentées essentiellement par le gène de fusion ALK-EML4 mais d'autres voies de la carcinogenèse sont également impliquées incluant HER2, BRAF, MET, RET…. Les plus grandes difficultés rencontrées dans le domaine de la génomique sont représentées par l'absence de réel consensus concernant les stratégies thérapeutiques, l'absence de techniques de diagnostic fiables et l'apparition inévitable de résistances secondaires impliquant de nouvelles voies de la carcinogenèse. Dans cette mise au point, nous présentons les voies de la carcinogenèse les plus explorées et ciblées ainsi que les stratégies diagnostiques adoptées en routine.

Mots-clés
Cancer du poumon, EGFR, ALK-EML4, KRAS

SUMMARY

Lung cancer is the first cause of death by cancer worldwide. In Tunisia, its incidence has increased from 17.6 cases per 100.000 persons in 1997 to 27.6 cases per 100.000 persons in 2003. Its prognosis has been improving thanks to the emergence of molecular targets. The first one is represented by EGFR (Epidermal growth factor receptor), which marks this year (2014) its tenth anniversary. Many other targets have been identified. The most famous and useful of them is the fusion gene ALK-EML4 but other oncogenic pathways have been implicated and are under investigations including HER2, BRAF, MET, RET…. The most relevant challenges encountered are represented by the difficulty to achieve a consensual decisional and therapeutic algorithm, the absence of standardized diagnostic techniques and the unavoidable occurrence of secondary resistance due to the activation of other oncogenic pathways that must be explored and targeted. In this update, we tried to present the major pathways implicated and the most relevant practice routine strategies.

Key-words
Lung cancer, EGFR, ALK-EML4, KRAS

Lung cancer is the first cancer in men and the leading cause of cancer-related death worldwide due to a diagnosis almost delayed. For researchers and clinicians who work in the field of lung cancer, recent genomic findings are considered as a storm according to Thomas Hudson, the president of the Canadian research institute (Ontario Institute for Cancer Research) during the colloque ProCaRT organized by the national institute of cancer (INCa) on the 14th January 2013 in France. In fact, many articles have been published dealing with genomic research and techniques of sequencing that are increasing and following an ascendant curve, which is more spectacular than the Moore's law (1). All these advances make us wonder about their benefit to the patient. Is it necessary to step back and identify the best strategies to adopt and the most useful markers to detect?

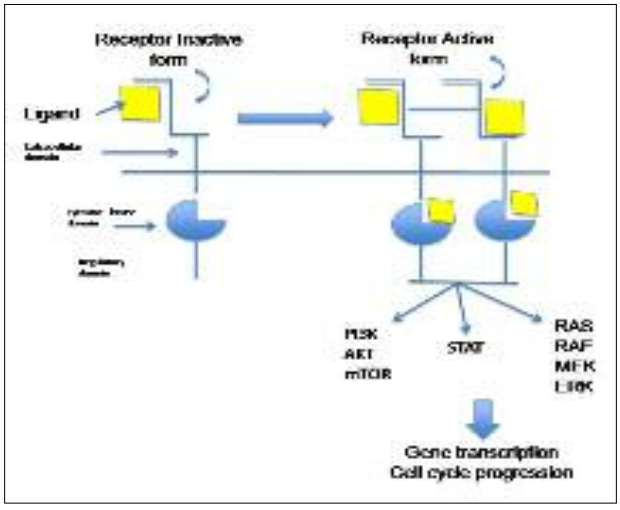
We performed an update based on a review of the literature searching for the most useful genomic markers in lung cancer. We performed this review in the pubmed using the key-words: genomic of lung cancer, personalized therapy in lung cancer and targets in lung cancer. Thousand of articles have been published so far. We tried to retain the most recent ones and to focus on the most useful molecular targets.

The most relevant molecular targets

Based on the literature findings, recent studies estimate that approximately 50-60% of patients with non small cell lung cancer (NSCLC) harbor at list one activated pathway with the most common mutations being in the Kirsten ras (KRAS) gene (24%) and the epidermal growth factor receptor (EGFR) gene (13-22%), with translocations involving anaplastic lymphoma kinase (ALK) in another 5-6% (1, 2). Other pathways are also explored including HER2Neu, BRAF.

* EGFR pathway: This year, 2014, marks the tenth anniversary of the discovery of somatic mutations of the EGFR gene in NSCLC. The discovery of activating mutations in the kinase domain of the EGFR gene has determined a revolution in the diagnosis, classification and management of these tumors. EGFR gene encodes for a transmembrane tyrosine kinase. Upon binding to its ligands, EGFR forms homodimers or heterodimers with other family members (ERBB2, ERBB3 or ERBB4), which inactivate intrinsic receptor tyrosine kinase activity and trigger a phosphorylation cascade of specific tyrosine residues within their cytoplasmic regulatory domains (3). These phosphorylated tyrosine residues activate several signaling pathways including mitogen-activated protein kinase (MAPK) pathway, phosphatidylinositol 3-kinase (PI3K/AKT pathway) and the signal transducer and activator of transcription pathways (Figure 1). The mutations of EGFR reach 40% in East Asians and 15% in Caucasians (4). The presence of an EGFR mutation predicts likelihood of response to TKI therapy, with an observed response rate of about 80% among individuals whose tumors harbor the mutation and only 10% among those whose tumors do not (5, 6).The most frequent mutations have been identified in exons 18, 19, 20 and 21. These mutations or deletions, mainly in exon 19, result in increased malignant cell survival, proliferation, growth, invasion, metastatic spread and tumor angiogenesis (7). These activating mutations are more frequently observed in never smokers female with Asian ethnicity and an adenocarcinoma histologic subtype. These mutations are detected in tissue and lung fluid. Many diagnostic methods have been described including Sanger sequencing, pyro sequencing, and next generation sequencing. The identification of mutations induces the use of target therapies. Gefitinib and erlotinib represent the first generation of small EGFR tyrosine kinase inhibitors that selectively target the intracellular tyrosine kinase domain of EGFR, blocking the downstream signaling of the receptor. Unfortunately, the use of these molecules raises many questions concerning the varying response in patients with lung cancers with sensitive EGFR mutations, the occurrence of resistance after a promising initial response, the most appropriate treatment schedule, the treatment strategies after acquisition of resistance to gefintib/erlotininb, the use of EGFR-TKI in adjuvant setting after surgical resection? Some patients with sensitive mutations don't respond well to the treatment. This has been reported to an inactivation of PTEN resulting in an activation of the PI3K pathway (8). Other authors reported the amplification of the proto-oncogene MET or the BCl2-interacting mediator of cell death (BIM) (9). All responders eventually develop resistance, most commonly because of the emergence of a gatekeeper mutation in the kinase domain, such as T790M in EGFR-mutated NSCLC or amplification of mesenchymal-epithelial transition factor (c-Met) (10, 11). Based on these data, several ongoing trials are assessing the efficacy of novel small molecule EGFR inhibitors for NSCLC including Afatinib or other EGFR/HER inhibitors.

Figure 1 : EGFR and KRAS pathways: The activation of the EGFR receptor needs a dimerization which is induced by the fixation of the ligand in the extra-membranous part of the receptor or its intracellular kinase domain.



* KRAS pathway

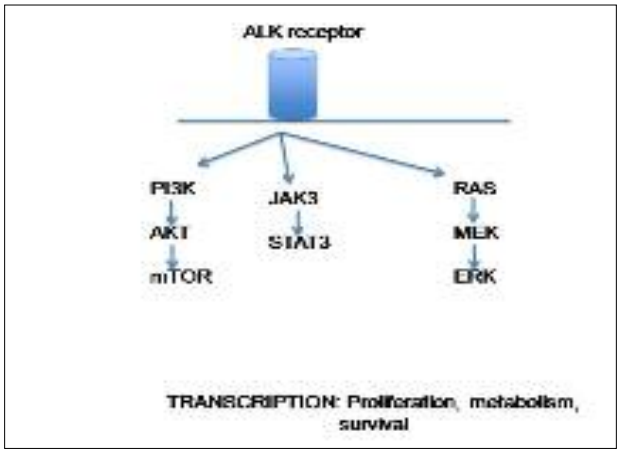
The activation of EGFR induces a cascade phosphorylation of RAS (rat sarcoma viral oncogene), RAF (v-raf murine leukemia viral oncogenehomolog), MEK (murine thymoma viral oncogenehomolog), ERK (extra cellular-signal-regulated kinase), PI3K/AKT (phosphatidylinositol 3-kinase). These interactions induce proliferation, neo-angiogenesis and metastasis (12). In the opposition to colon cancer where the negativepredictive value of these mutations on the response to EGFR-TKI has been proved, the impact of these mutations in NSCLC is still debated with contradictory results (13). In the opposition to the EGFR pathway, which is implicated in non smokers, KRAS pathway is activated in smokers with

adenocarcinoma. Few trials concerned KRAS mutations. This may be due to the inactivation of the enzymatic activity of KRAS in case of mutations. This fact induces the inefficacy of the treatment. This finding puts emphasis on the necessity of combining the therapeutics with inhibitors of other pathways including PI3K and MEK pathways (14), Serine threonine kinase 11 (STK11), NF1pathway, WT1 pathway, NK-KB, GATA-binding factor 2, RNA-binding Motif 5, Il8, Twist-related protein 1(15, 16, 17, 18)….

* ALK pathway

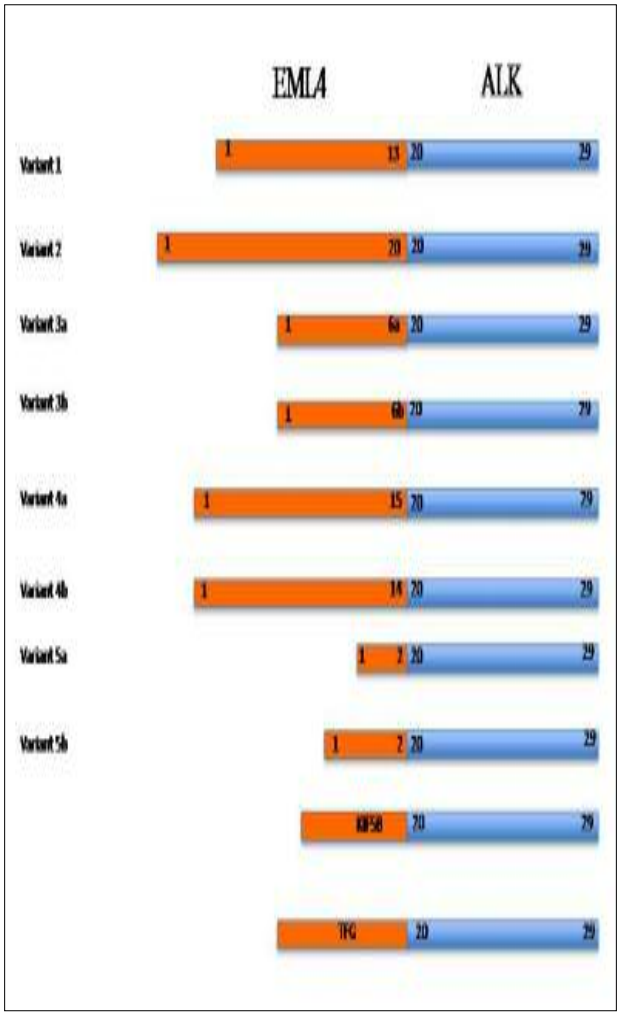
The anaplastic lymphoma kinase gene (ALK) is located on chromosome 2p23. This gene was originally established for its implication in the pathogenesis of inflammatory myofibroblastic tumor and ALK-positive anaplastic large-cell lymphomas (19). The fusion of ALK with the echinoderm microtubule-associated protein-like 4 gene (EML4) was initially identified in 2007 (19). This gene fusion is due to small inversions on chromosome arm 2p. Further publications reported other partners to ALK gene including TGF gene located at 3q12.2 and KIF5B located at 10p11.22 (20, 21). The ALK pathway and its relevant interactions are represented in figure 2.

Figure 2: ALK pathway



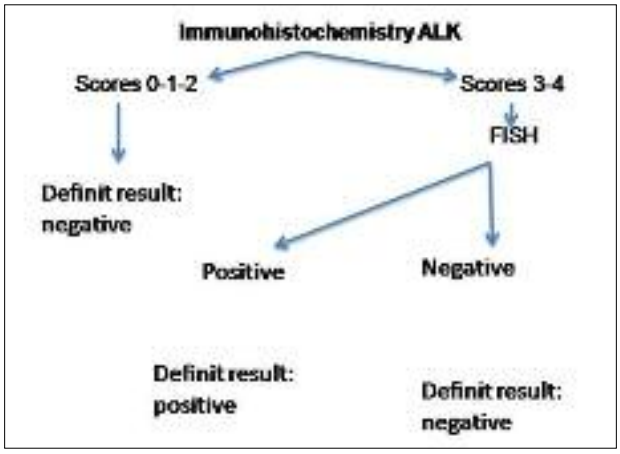
At least eleven variants of the EML4-ALK fusion gene with the same breakpoint in ALK gene and different ones in EML4 gene have been reported. The different variants are represented in figure 3. The most common variants are E13, A20 and E6a/b. Variant A20, 1 and 3a/b have been detected in 33% and 29% of NSCLC with ALK-EML4 translocation (19). The EML4-ALK gene fusion results in the overexpression of the ALK protein. It was stipulated that ALK rearrangement is mutually exclusive with such other mutations as EGFR or KRAS (22) but recent evidence suggests that coexisting ALK and EGFR mutations can happen before any targeted treatment (19, 23). In opposition to the EGFR mutations, the fusion gene is generally observed in less than 5% of all NSCLC in relatively young non-or-light smokers. Histologic subtype is an indicator of such a fusion. In fact, the translocation is mainly observed in adenocarcinoma with solid signet-ring cell and mucinous cribriform patterns (24). Many investigation methods have been reported to assess the ALK-EML4 translocation including polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH) and immunohistochemistry. All these techniques may cause several pitfalls. FISH was the first diagnostic technique for detecting ALK fusion (25).

Figure 3 : Variants of fusion gene ALK-EML4: At least eleven variants have been reported with E13, A20 and E6a/b being the most frequent (19).



This technique has limitations for use in routine practice because it is time consuming and expensive besides it may be confusing in case of polysomy (26). RT-PCR is a rapid and highly sensitive method. In order to target all the potential transcripts, this method must be multiplexed. The limits of this technique consist in the necessity of a cryopreservation of tumor samples in order to obtain an available RNA and in this way, it is difficult to apply to archival tissues (25, 27). Immunohistochemistry has the advantage of being a cheap, easy-to-use and routine technique. Several antibodies are available with different sensitivities. This technique is used as a tool of screening with a high negative predictive value (28). Agreement between the 3 techniques is poor and variable according to the EML4-ALK variant (29).

Figure 4 : Decisional diagram for the screening the ALK-EML4 fusion gene.

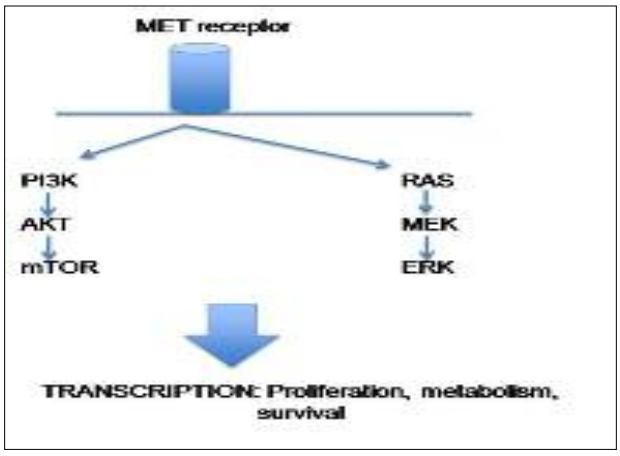


The figure 4 is an example of decisional algorithm concerning the detection of ALK-EML4 translocation according to the literature. The ALK protein kinase is the target of crizotinib. The dilemma encountered since the commercialization of this treatment is the occurrence of resistance. This resistance has been reported to be related to secondary mutations in the ALK tyrosine kinase domain, ALK copy number gain and the presence of another oncogene driver (EGFR or KRAS) (30, 31, 32, 33). During the 2012 ASCO meeting in Chicago (USA), Doebele suggested a subdivision of resistant tumors into those having persistent ALK pathway dominance (secondary mutations in the ALK tyrosine kinase domain with or without increased gene copy number) and those with ALK non-dominant pathway (other oncogene driver, loss of the ALK translocation) (34).

* MET pathway

MET (mesenchymal-epidermal transition axis) is a receptor tyrosine kinase that has been under intensive preclinical investigation for over 25 years. MET is now known to be a new "druggable" target within the human kinome with promising results in NSCLC. MET pathway interacts with MAPK pathway, PI3K-mTOR and STAT pathways that are implicated in cell proliferation and angiogenesis (Figure 5) (35, 36).

Figure 5 : MET pathway

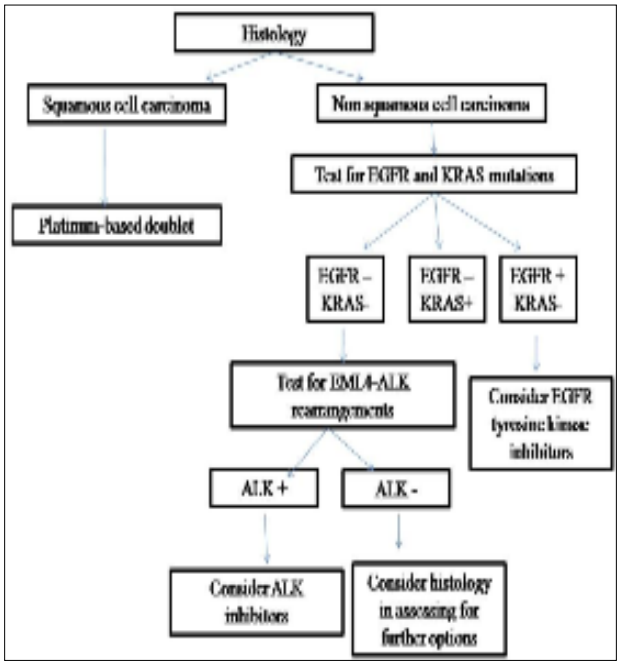


MET is also known to crosstalk with a number of other signaling pathways (37) including EGFR/ERBB family receptors and KRAS signaling (38, 39). MET signaling can be altered through ligand or receptor overexpression, genomic amplification, mutations or alternative splicing. MET receptor is often over-expressed in NSCLC (40). MET amplification has been reported in 2 to 21% of NSCLC lung adenocarcinomas particularly in TKI-naïve cohorts (41, 42). The prognostic impact of MET amplification is controversial with a negative prognostic impact in the study of Cappuzzo and coworkers (43) and a positive prognostic relevance in the study of Kanteti and colleagues (44). MET gene amplification is reported mainly in EGFR-TKI resistant cell clones (45) and more rarely in untreated EGFR-mutant patients with respectively 22% and 3% of the patients (45, 46). On the other hand, 44% of patients found with MET amplification had concurrent T790M EGFR TKI-resistant mutation. There are at least four possible strategies for inactivating HGF/MET pathway with HGF binding to MET, anti-MET monoclonal antibodies, small molecule MET kinase inhibitors and small molecule downstream pathway inhibitors of STAT3 (47, 48). There is no consensus about an algorithm for personalized MET targeted lung cancer therapy because many interrogations have to be resolved concerning the target patient group that would benefit from the treatment, the best diagnostic methods, the use as a single agent or in combination with other targeted agents and finally the drugs to use in case of potential acquired resistance which seems to be unavoidable in the field of target therapy. Many phase I, II and III clinical trials of anti-MET agents are currently under investigation.

Many other treatments will represent future alternative therapeutics targeting HER2, BRAF, RET, PI3K/AKT/mTOR pathway but no consensus concerning their use has been achieved (49).

According to the recent findings, the most consensual diagnostic diagram to search for molecular targets and consequent management is represented in figure 6.

Figure 6 : Decisional diagnostic diagram of molecular targets and consequent management.



CONCLUSION

A personalized medicine necessitates an accurate histologic diagnosis of tumors. Dr Rhénanie from Nord-Westphalie presented in the international congress about lung cancer held in Sydney (Australia), a study conducted between 2010 and 2013 about 5,000 patients with lung cancer. A genomic classification allowed obtaining an accurate histological diagnosis (50). Besides, this genomic cartography allowed a personalized treatment. In fact, patients treated with TKI-treatment or anti-ALK treatment presented a mean benefit of survival reaching 2 years in comparison with those treated by conventional chemotherapy. This study was possible thanks to a unique collaboration between the universitary hospital and the University of Cologne into 2 projects: The "Clinical Lung Cancer Genome Project (CLCGP)" and the "Netzwerk Genomische Medizin (NGM)". These projects were handled by the BMBF, into 2 programs: NGFN-Plus andPerMed.NRW.

This update about the genomic of lung cancer made us consider the importance of 2 factors: the necessity of focusing on molecular biology and the importance of a narrow collaboration between clinicians, molecular pathologists and scientists.

Focus on molecular biology

Recent decades showed important advances in sequencing human genome identifying genes implicated in increased cancer risk. Besides, the rapidity of the commercialization of target therapies makes us optimistic toward the prognosis of lung cancer. The best example of the latter phenomenon is the commercialization of the crizotinib which targets ALK-EML4 translocation only four years after the first identification of the fusion gene in comparison to the imatinib the treatment of chronic myeloid leukemia, which was commercialized 40 years after the initial description of the chromosomal abnormality. The bad news of target therapies is the unavoidable phenomenon of secondary resistance which has to be anticipated, elucidated and targeted by new drugs.

The necessity of a narrow collaboration

The molecular abnormalities are particular to each organ. This fact made an international collaboration mandatory in order to obtain a homogeneous series for clinical studies (51). This collaboration implicates the constitution of tumor banks and biologic resources (52). The integration of the patients in such projects causes ethical and social dilemma whose resolution depends on empiric researches focused on the real needs and patients' expectations (52). The solutions will not be politically robust unless there will be a collaboration with researches in social sciences in order to guarantee an equal access to personalized medicine. A European project co-directed by B. Prainsack will be published in the further months (53).

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