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Heterogeneity in Lung Cancer

Vitor Manuel Leitão de Sousa[a–d] Lina Carvalho[a–d]

[a]Institute of Anatomical and Molecular Pathology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [b]CIMAGO – Research Center for Environment, Genetics and Oncobiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [c]Centre of Pulmonology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; [d]Service of Anatomical Pathology, University Hospital of Coimbra, Coimbra, Portugal

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Abstract
Lung cancer diagnosis is a challenge since it is also one of the most frequently diagnosed cancers. Diagnostic challenges are deeply related to the development of personalized therapy and molecular and precise histological characterizations of lung cancer. When addressing these features, it is very important to acknowledge the issue of tumour heterogeneity, as it imposes several questions. First of all, lung cancer is a very heterogeneous disease, at a cellular and histological level. Cellular and histological heterogeneity are addressed with emphasis on the diagnosis, pre-neoplastic lesions, and cell origin, trying to contribute to a better knowledge of carcinogenesis. Molecular intra-tumour and inter-tumour heterogeneity are also addressed as temporal heterogeneity. Lung cancer heterogeneity has implications in pathogenesis understanding, diagnosis, selection of tissue for molecular diagnosis, as well as therapeutic decision. The understanding of tumour heterogeneity is crucial and we must be aware of the implications and future developments regarding this field.

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Introduction

Lung cancer is one of the most frequently diagnosed cancers, especially in developed countries. Despite recent developments in the diagnosis, classification, and therapy, the overall survival is still poor. Better understanding of the pathology of these tumours, especially molecular pathology, is necessary to accumulate knowledge in order to better address this issue, aiming at personalized therapy.

Tumour heterogeneity has been described in several tumours and it has been addressed in several ways: histological, cellular, and molecular/genetic. Lung cancer constitutes a group of heterogeneous tumours, with several differentiation types, recognized by the WHO classification of lung tumours. In this classification, the importance of molecular characterization of lung cancer is recognized.

Tumour heterogeneity has an important impact not only on tumour classification but also on defining prognosis and therapy decision. So, it is crucial to understand the implications of tumour heterogeneity in daily diagnosis routine.

The authors aim to address tumour heterogeneity in lung cancer, exploiting histological, cellular and molecular heterogeneity. They intend to review the heterogene-

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E-Mail karger@karger.com
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Vitor Manuel Leitão de Sousa
Instituto de Anatomia Patológica, Faculdade de Medicina
Universidade de Coimbra, Rua Larga
PT–3000-054 Coimbra (Portugal)
E-Mail vitorsousa.patol@gmail.com

ity issue, discussing and reflecting on lung heterogeneity and addressing the new WHO classification and several published knowledge.

Histological and Cellular Heterogeneity in Lung Cancer

Lung cancer is a heterogeneous group of cancers. Improvements in the histological classification are continuously being made. The new WHO classification of lung tumours has recently been published. This classification takes into account the great histological heterogeneity in lung cancers. It recognizes several types of lung cancers, such as epidermoid carcinomas, adenocarcinomas, small cell lung carcinomas, large cell carcinomas, large cell neuroendocrine carcinomas, adenosquamous carcinomas, sarcomatoid and pleomorphic carcinomas, and several other types. Non-small cell lung cancer reporting is not acceptable without an immunohistochemical profile to achieve differentiation as well as to identify genetic alterations and biomarkers, some with predictive value for therapy decision. Small biopsies are prone to identify CK7/TTF1, CK5.6/p40, and vim/neuroendocrine markers. Biopsies keep being the most representative tissue to classify and treat pulmonary carcinomas due to the high percentage – 70% – of tumours diagnosed in a non-surgical way.

This great variety of histopathologic diagnosis reflects tumour heterogeneity that could be explained by different cells of origin or differentiation pathways.

Even in carcinomas like squamous cell carcinomas or adenocarcinomas heterogeneity has been found, as histological subtypes have been defined. For instance, variants of squamous cell carcinoma are recognized. The same is true for adenocarcinomas. These histopathological subtypes have diagnostic, prognostic, therapeutic and demographic distinct features. So, although similar in differentiation, they represent different tumours, thus reinforcing that heterogeneity is a feature of these tumours.

Histological and cellular heterogeneity in lung cancer is also well proven when we look for tumours with more than one type of differentiation. Adenosquamous carcinomas are a good example. They clearly demonstrate heterogeneity at the cellular level as we can find cells with adenocarcinoma differentiation markers like CK7 and TTF1 as well as with squamous differentiation markers such as CK5/6 or other high-molecular-height cytokeratins. This is also true for pleomorphic carcinomas where we can find areas with squamous or adenocarcinoma differentiation and giant and/or fusiform cells.

Cellular heterogeneity is also observed in combined tumours such as small cell lung carcinomas combined with other lung carcinomas such as adenocarcinoma or squamous cell carcinoma or large cell neuroendocrine carcinomas combined with other carcinomas.

This cellular heterogeneity could be, at least in part, explained by different cell origins. Pre-neoplastic lesions for some lung carcinomas are defined.

Two repair/carcinogenic pools of adult stem cells have been reported and are related to pulmonary carcinoma heterogeneity: the TRU – terminal respiratory unit; i.e., the respiratory bronchiole and adjacent alveolar duct/septae – and the adult central respiratory epithelium till the TRU. Embryonic development of human-induced pluripotent stem cells after induction of adult fibroblasts in mouse skin has demonstrated the whole embryonic potential informing about the repair/adaptation and the possibilities of the intermingling of meso/ecto/endoderm in one single cell. In this preliminary study, CK7, CK5.7, TTF1, VIM, CD56, and Ki-67 demonstrated to be enough to classify carcinomas according to cellular populations/heterogeneity. Similar results have been obtained with chromium treatment of both fibroblasts and normal bronchial cells in contact where epithelial-mesenchymal transition was revealed.

It is well known that squamous cell carcinomas have a basal cell origin (in the respiratory epithelia), demonstrated by our group and several other investigators. The sequence basal cell hyperplasia – squamous metaplasia – squamous dysplasia – squamous carcinoma is well defined. Our research group also demonstrated molecular changes in this spectrum of lesions, reflecting the severity and biological aggressiveness along the spectrum of lesions, using immunohistochemical markers such as Ki-67, p53, EGFR, and HER2 and also evaluating EGFR and HER2 gene copy number [1].

We have studied EGFR expression in these pre-neoplastic lesions, demonstrating its increasing expression in lesions in this spectrum; therefore, EGFR and its respective pathways may play a role in early steps of epidermoid carcinoma development, reflecting the importance of EGFR signalling transduction pathways in pre-neoplastic lesions [1]. Other studies also corroborate these results [2–9]. On the other hand, it seems that HER2 might not be involved in the first steps of epidermoid cancer development; other studies have found identical results [8, 10, 11].

EGFR, Ki-67, and p53 might play a role in the identification of epidermoid carcinoma pre-neoplastic lesions at higher risk of developing epidermoid carcinoma [1].

An increasing expression of these markers was observed [1]. The study reinforced the utility of Ki-67 as a biomarker for dysplasia, a group of pre-neoplastic lesions characterized by a higher proliferative index, also identified by other authors [1, 12–17].

Atypical adenomatous hyperplasia is considered as an adenocarcinoma pre-neoplastic lesion. Peripheral adenocarcinomas have their origin in epithelial cells of the TRU, like pneumocytes, with CK7 and TTF1 expression. Nevertheless, we must consider that more central adenocarcinomas have a different cell of origin as they develop upstream the TRU. So, it is hypothesized that the origin is in cylindrical cells of the respiratory tract. Also, adenocarcinomas can clearly have a lung differentiation demonstrated by CK7 and TTF1 positivity, but some lung adenocarcinomas express CK20 and are TTF1 and CK7 negative, revealing a colonic-like expression. Another perspective is to observe the way cells are organized. Several patterns of cellular organization can be observed. These different patters may reflect not only cellular type/differentiation but molecular differences that could explain cellular organization and even biological behaviour, as well as prognostic and therapeutic differences in between patterns. Our research group has been addressing lung carcinomas considering histological patterns, especially in adenocarcinomas but also exploring adenosquamous and pleomorphic carcinomas [18]. For instance, we proved the relevance of pattern classification demonstrating that adenocarcinomas are molecularly different from normal adjacent tissue, and that acinar and BA/lepidic patterns are the most alike and papillary patterns the most different [18]. Cluster analysis revealed three clusters: papillary, solid, and a group composed of acinar, BA/lepidic and micropapillary patterns [18]. Papillary and solid patterns revealed lower TTF1 expression (identical to normal tissue), exhibiting a non-TRU/bronchial phenotype [18]. Acinar, BA/lepidic and micropapillary patterns showed higher TTF1 expression corresponding to TRU origin [18]. These patterns, especially lepidic and acinar, being TTF1-positive, are those where EGFR mutations are said to be more frequent [18]. TTF1 expression, identifying possible TRU origin, defines a subgroup of adenocarcinomas with molecular and biological particularities [18]. The solid pattern also revealed lower HER2 and higher EGFR and ERCC1 expression (compared to papillary) [18]. In solid patterns, EGFR pathway activation was related to EGFR overexpression [18]. We stated that adenocarcinomas with a solid pattern were less differentiated adenocarcinomas, with a worse prognosis [18]. Papillary patterns showed higher HER2 and lower ERCC1 expressions. It seems that impaired DNA repair mechanisms are implicated in carcinogenesis when the papillary pattern is dominant [18]. Adenocarcinomas showed higher TTF1 expression in acinar, BA/lepidic and micropapillary patterns corresponding to TRU adenocarcinomas that express TTF1 [18, 19], probably related to a better prognosis when compared to non-TRU-type adenocarcinomas [20]. This is also highlighted by some authors that identified an inverse correlation between TTF1 and Ki-67, a marker of proliferation and biological aggressiveness [21]. We identified higher TTF1 expression in acinar and lepidic patterns, in TRU-type adenocarcinomas, corroborating the published results [18]. TTF1, expressed in TRU-type adenocarcinomas, has been associated with good prognosis [21–26].

However, Pelosi et al. [27] did not find a correlation between TTF1 expression and prognosis. Several studies identified a correlation between TTF1 expression, like TRU-type adenocarcinomas, and EGFR mutations [28, 29]. TRU-type adenocarcinomas have also been associated with EGFR mutations [28–32]. It is also known that EGFR mutations are more frequent in lepidic and acinar patterns. EGFR protein expression has been described as more frequent in TRU-type adenocarcinomas as well as in epidermoid lung carcinomas [33].

The micropapillary pattern had higher retinoblastoma protein (RB) expression, and the acinar pattern lower ERCC1 and higher EGFR expression when compared with normal tissue [18]. Cyclin D1 seemed to be relevant in acinar and BA/lepidic patterns and not related to the micropapillary pattern [18].

ERCC1 protein expression in micropapillary, solid and BA/lepidic patterns indicated DNA repair preservation, while in acinar and papillary patterns, there was lower expression [18]. Lung cancer with higher ERCC1 expression was associated with cisplatin-based chemotherapy resistance as ERCC1 acts by removing DNA adducts, which relates to poor prognosis [34–39].

These differences identified between the adenocarcinoma patterns represent a form of heterogeneity with implications in the diagnosis, pathogenic understanding, and therapeutic outcome.

We can also argue that carcinogens could have a role in determining heterogeneity as some lung carcinomas are more frequently diagnosed in smokers or ex-smokers, such as small cell lung carcinoma and squamous cell carcinoma, and some other carcinomas, like adenocarcinoma, are being diagnosed in never-smokers. However, there are adenocarcinomas related to smoking and others not related to smoking. This fact indicates that the envi-

ronment is also a determinant of tumour heterogeneity, as different exposure histories are related to different genetic and molecular changes, explaining the histological heterogeneity. Also, we know that several lung pathologies/conditions are associated with an increased risk of lung carcinoma, such as lung fibrous/scar areas and lung interstitial diseases. These diseases could constitute a field of cancerization, and some may share molecular pathways with the lung cancers, such as pathways implicated in epithelial mesenchymal transition (EMT).

Tobacco and professional or other exposure inhaling demand cellular remodelling and adaptation of the basal cells in either TRU or respiratory epithelium, with consequent hyperplasia of vimentin-positive cells and TTF1 bronchial-positive cells, demonstrated in carcinomas arising after molecular transformation in less matured cells, and consequently, in multi-patterned carcinomas and in pleomorphic carcinomas. This approach facilitated pulmonary carcinoma classification in biopsies but does not correlate directly with metastatic potentiality.

Histological heterogeneity is also observed in the differentiation grade. For instance squamous cell lung carcinoma could be classified as well or poorly differentiated. The degree of differentiation is associated with a greater biological aggressiveness and poor outcome.

So, only by looking for cellular and histological heterogeneity can we say that lung carcinomas are a good model for cancer heterogeneity study. Cancer heterogeneity is a constant and identified in other organs and system of organs.

Several authors recognize morphologic heterogeneity in lung adenocarcinomas and the WHO recommends reporting the patterns present as well as the most prevalent pattern. These patterns have prognostic value, with the lepidic pattern having better prognosis and the micropapillary and solid pattern having worse prognosis. This morphologic heterogeneity also has therapeutic implications as patients with solid and micropapillary patterns benefit more from adjuvant chemotherapy [40–42].

We also know that different molecular expression patterns are associated with heterogeneity as well as with prognosis. The differential expression of several molecular markers, such as c-erb-2, bcl-2, p53, p63, rb, egfr, and neuroendocrine markers, reinforces the utility of pattern-based classification [40, 41].

Morphologic heterogeneity has clear prognostic implications. We clearly know that the histological type is associated with prognosis (for instance small cell carcinoma has worse prognosis and in situ adenocarcinoma and typical carcinoid have better prognosis). Also, the histological subtype is associated with prognosis (for instance, well-differentiated squamous cell carcinoma compared to basaloid squamous cell carcinoma and typical carcinoid compared to atypical carcinoid) [42–45].

We all also recognize that morphologic or histologic heterogeneity has therapeutic implications especially as the prevalence of molecular targetable changes differs in between histologic subtypes [42, 45, 46]. For instance, *EGFR* mutations are more frequently identified in lung adenocarcinoma, especially arising from the TRU, and also more frequently identified in some patterns like lepidic, papillary and acinar patterns [45, 47]. *ALK* translocations are more prone to be identified in lung adenocarcinomas and in acinar, solid and signet cell morphology [45, 48–50]. However, there are some conflicting results as some authors identify *EGFR* mutations in all the patterns of lung adenocarcinomas; these authors identified an association of *KRAS* and *BRAF* mutations and high nuclear grade [51].

Molecular heterogeneity has been identified in morphologic heterogeneous carcinomas such as in adenosquamous cell carcinomas, for *KRAS* mutations and *EGFR* mutations [52]. Molecular heterogeneity related to *KRAS* mutations status has also been identified in pleomorphic carcinomas [53]. By whole-genome sequencing, different rates of gene mutations, gene copy number alterations, and different protein expression levels and protein phosphorylation levels were demonstrated, confirming the association between morphologic and molecular heterogeneity [54]. Fang et al. [54] reported that p53 is the most frequently mutated gene; *KRAS*, *EGFR*, *MLL3,* and *STK11* the most frequently mutated genes in adenocarcinomas; *PI3KCA*, *SOX2*, *CDK2*, *P63,* and *FGFR1* the most frequently mutated genes in squamous cell carcinomas; and *RB1*, *MLL2*, *SMO,* and *PI3KCA* the most frequently mutated genes in small cell lung cancer.

Molecular and Genetic Heterogeneity

Tumour molecular and genetic heterogeneity has been identified not only in lung tumours but also in other organs. Molecular/genetic heterogeneity has been identified in breast, gastric, bladder, prostate, pancreatic, as well as in lung cancer [55–57].

Only 1/3 of somatic mutations are present in all the regions of the same tumour [57]. Intra-tumour heterogeneity (ITH) was first described by Fidler [58].

Genetic heterogeneity has been described in lung cancer [59–63]. Tumour heterogeneity has been explained by

genetic heterogeneity (intra- and inter-tumour heterogeneity) and also by non-genetic heterogeneity driven by external and internal pressures allowing outgrowth of cell subpopulations, some cases depending on selective pressure related to microenvironment and interactions with immune and stromal cells or with matrix components [61, 62, 64, 65].

Molecular heterogeneity could be explained by several mechanisms such as by stem cell theories, by genomic or chromosomal instability, epigenetics modifications, and by adaptation mechanisms in response to microenvironment stimulus [66]. This molecular/genetic heterogeneity is associated with resistance to therapy like EGFR-targeted therapy [66].

ITH is also explained by clonal (monoclonal or polyclonal) evolution resulting in genetic heterogeneity, by selective pressure induced by the microenvironment or by chemotherapy favouring one or more than one clone of cells, by the EMT theories and by inter-clonal cooperation mechanisms [61, 67–69]. Inter-clonal cooperation is also important for metastases, as clones favour metastases by sequential or simultaneous cooperation in motility, matrix degradation, vascular invasion or distant colonization [67, 70–74].

Other authors associate tobacco with ITH, as they identified *EGFR* mutation heterogeneity according to adenocarcinoma pattern (more frequent in lepidic pattern) and to tobacco habits [75]. Multiple carcinogens present in tobacco are related to the development of a field of cancerization, where cells tend to accumulate several molecular changes leading to genetic instability and therefore favouring heterogeneity. Other authors identified associations between gene copy numbers (for *EGFR*) and intra-tumour mutation heterogeneity [76]. These authors propose that non-small cell lung cancer could be stratified into four groups according to ITH: pure mutated; pure wild-type, mutated heterogeneous; and wild-type heterogeneous. Those tumours with higher or pure mutational rates mutated with better response to EGFR-targeted therapy [76].

DNA repair has important implications in cancer. Deficient DNA repair function allows genomic instability [77, 78]. Genomic instability allows the accumulation of mutations thus promoting heterogeneity. DNA repair impairments could also explain the increased sensitivity of tumour cells to radiation and chemotherapy and thus clonal selection pressure [77, 78]. DNA damage could be related to spontaneous hydrolysis, cytosine deamination, mismatched bases, and secondary to reactive oxygen species (ROS) [77, 78]. Also, anticancer agents, such as alkylating agents and bleomycin, are responsible for DNA breaks [77, 78]. Six different mechanisms are involved: mismatch repair, homologous recombination and non-homologous end joining, translesion DNA synthesis, base excision repair, and nucleotide excision repair [77, 78].

Several authors and studies revealed molecular/genetic heterogeneity concerning *EGFR* and *KRAS* mutations; however, conflicting results have been published [79]. Bai et al. [76] identified an ITH rate of 28.2% (24/85%); Mansuet-Lupo et al. [80] identified an ITH rate of 5% (2/40); Kim et al. [81] an ITH rate of 2.9% (1/34); Tomonaga et al. [75] an ITH rate of 23.7% (9/38); Taniguchi et al. [82] an ITH rate of 28.6% (6/21); Zhang et al. [83] an ITH rate of 100% (7/7); Zhong et al. [79] found an ITH rate of 15.4% (10/65), mainly in adenocarcinomas and in some studies in squamous cell carcinomas and adenosquamous carcinomas. On the other end, other studies and authors did not find ITH [29, 84–86]. Some of these works had small samples and studied less foci of the same tumour compared to the studies where ITH was identified. Other authors showed that *EGFR* mutation heterogeneity is rare in primary tumours and metastasis, being higher in multiple lung nodules, with ITH rates of 9.1%, discordance rate between primary and lymph node metastasis of 10.2%, discordance rate of 14.3% between primary and distant metastasis and discordance between multiple lung nodules of 24.4% [87].

Zito Marino et al. [88] identified ITH for *ALK* translocation but no ITH for *EGFR* in lung adenocarcinomas.

Several authors (Badalian et al. [89], Cortot et al. [90], Kalikaki et al. [91], Schmid et al. [92], and Sun et al. [93]) identified ITH for *KRAS* mutation status in adenocarcinomas, ranging from 69 to 86%. However, Alsdorf et al. [94] reported that intra-tumour *KRAS* heterogeneity is a rare event, without discordance between primary tumour and metastasis.

For *MET* high intra-tumour spatial heterogeneity rate was identified in non-squamous lung carcinomas, associated with worse prognosis [95].

Taking into account that most of the authors report intra-tumour genetic/molecular heterogeneity, we could ask if the small biopsy is representative of the whole genetic panorama of the neoplasia. As the concordance rate varies between 68 and 97.1% for *EGFR* mutation status, we could argue that according to our group reports for most cases biopsy tissue will be representative, but not in some other cases [75, 76, 79–83, 96]. On the other end, Zhong et al. [79] and Yatabe et al. [84] stated that EGFR, KRAS, and ALK ITH is rare, defending the representativeness of the tissue obtained by biopsy. Mansuet-Lupo et al.

[80] also showed that *EGFR* mutations are independent of the primary tumour localization, of the type of sample, and consistent between primary and metastasis, validating the use of biopsies. Intra-tumour genetic heterogeneity for *EGFR* or *ALK* could also explain different TKI response rates between patients. Clonal selection and acquisition of new mutations could also be responsible for resistance. Re-biopsy or metastasis biopsy is important to evaluate therapeutic resistance. As some patients do not have conditions to re-biopsy or have inaccessible tumours, liquid biopsy is gaining importance and actually is a valid tool for diagnosis, evaluation of response, and recurrence as well as to address spatial and temporal heterogeneity.

Temporal genetic/molecular heterogeneity is also being studied in lung cancer. This could be related with temporal heterogeneity in the primary tumour or between primary tumour and metastasis over time. This temporal heterogeneity is of great importance in the understanding of recurrence and therapeutic resistance. Kim et al. [97], by next-generation sequencing, identified infrequent genetic heterogeneity of 16 genes between primary tumour and metastasis. Sherwood et al. [98], in a review of 26 articles, demonstrated that there are variable discordance rates between primary tumour and their metastases. However, as there is a substantial concordance, the molecular diagnosis could be made in the primary tumour or in the metastasis, recommending, however, the use of sensible methods [98]. The concordance or discordance could be explained by sample issues as the percentage of tumour cells, by the methodologies applied on diagnosis, by ITH, temporal heterogeneity (mutational status evolution), and preservation methods and even by the local of metastasis [98]. EGFR concordance rate between primary tumour and metastasis ranged from 100 to 72% [98]. Kalikaki et al. [91] identified concordance rates of 72% (18/25 cases), Schmid et al. [92] 94% (90/96), Mansuet-Lupo et al. [80] 90% (9/10), Sun et al. [93] 91% (73/80), Wei et al. [105] 94% (47/50), Yatabe et al. [84] 100% (77/77), Shimizu et al. [104] 86% (60/70), Park et al. [103] 88% (89/101), Matsumoto et al. [102] 100% (8/8), Luo et al. [101] 93% (14/15), Gow et al. [99] 73% (49/67), and Han et al. [100] identified a concordance rate of 81% (30/37). KRAS concordance rate between primary tumour and metastasis ranged from 100 to 64% [98]. KRAS concordance rates identified by different authors were: 76% (19/25) [91]; 74% (71/96) [92]; 93% (74/80) [93]; 100% (9/9) [94]; 100% (15/15) [106]; 64% (7/11) [89]; 71% (15/21) [90]; 81% (17/21) [90] (using ARMS); and 97% (36/37) [100].

Lung cancer heterogeneity raises prognostic questions. Recurrence after therapeutic resistance is one of the most important causes of cancer-related death. Resistance mechanisms are related to signal transduction-redundant activation, new mutations, synergic interactions with the target gene, EGFR inhibition bypass, EMT phenotype acquisition, DNA hypermethylation, and also related to the emergence of new tumour cell subclones with secondary mutations resistant to previous therapy, this event is related to selective pressure and heterogeneity (cellular and molecular) [66, 107, 108]. Therapeutic selective pressure is associated with *EGFR* TKI resistance [66, 107, 108]. Chemotherapy and targeted therapies are associated with the reduction of the number of sensible cell clones and to a higher proportion of resistant clones that persists after treatment. After chemotherapy, the response rate to EGFR TKIs is lower and some authors report a decrease in *EGFR* mutation rate [76, 109, 110]. Chen et al. [87] also demonstrated higher intra-tumour and inter-tumour heterogeneity in tumours of patients submitted to chemotherapy. Resistance mutations and amplification rates are higher before EGFR TKIs [66]. EGFR TKIs resistance takes place about 10–13 months after EGFR TKIs, related frequently to *EGFR* T790M mutation, *MET* amplification, *HER2* mutation, and *KRAS* mutation. Anti-ALK therapy resistance is often related to ALK mutations and amplifications, KIT amplification and EGFR activation. Bai et al. [76, 109] showed that patients with tumours showing higher *EGFR* gene copy number had lower ITH and those patients with higher heterogeneity had a worse prognosis and survival rate. Also they stated that tumours with higher *EGFR* mutation rates showed better *EGFR* TKI response [76, 109]. Taniguchi et al. [82] also demonstrated that time to progression and overall survival after EGFR TKIs is significantly lower in patients with tumours with high ITH.

As expected intra- and inter-tumour genetic and molecular heterogeneity have important therapeutic implications. We must also take into account that molecular heterogeneity is also reflected when we consider that some genes could have different mutations or genetic events, some conferring different therapeutic sensibility, as described for *EGFR* mutations where we can also find resistance conferring mutations, like T790M *EGFR* mutation [111]. Other *EGFR* mutations are associated with less sensibility to TKIs (exon 18 G719 and exon 21 L861Q for instance), and exon 20 in frame insertion are associated with no affinity for TKIs [111].

ITH has also been identified at a metabolic level, some with prognostic significance and implications in disease progression after chemotherapy [112–115].

Another important issue is related to molecular heterogeneity when dealing with primary and metastatic disease. Several reports address this problem [58, 93, 98, 104, 116–118]. Temporal heterogeneity is also an issue to consider, with clinical implications in the clinical follow-up, determining the risk of recurrence and metastases, related to acquiring resistance to chemotherapy or targeted therapy [108, 109, 119–122]. Several authors have been addressing these issues.

To address the questions imposed by spatial and temporal heterogeneity including heterogeneity between primary tumour and metastasis and by the selective therapeutic pressure, several authors are recommending the sequential sampling of tumour cells or genetic material. This could be achieved by liquid biopsy either addressing circulating tumour cells or cell-free DNA like ctDNA. These approaches are useful in the selection of the treatment, monitoring and evaluation of early recurrence and minimal residual disease, and resistance acquisition [123–125]. This methodology is also relevant when patients show no tolerance to a new biopsy, if there are several metastases in different localizations, when the tissue is insufficient or has artefacts related for instance to decalcification, when there are problems related to heterogeneity or when biopsy imposes risks [124]. ctDNA can be used to identify resistance to TKIs [126–135]. Weber et al. [136] demonstrated a concordance rate of 90% (179/199) for *EGFR* mutations between biopsy tissue and ctDNA (plasma) before EGFR TKIs. These authors identified *EGFR* mutations in the plasma not identified in the biopsy, probably related to sampling issues or heterogeneity [136]. Douillard et al. [137] demonstrates a concordance rate of 94.3% between tumour samples and plasma ctDNA. Mok et al. [138] achieved a concordance rate of 88%. Kim et al. [139] identified a concordance rate of 87.7% and 5 cases (8.7%) with *EGFR* or *KRAS* mutations only in plasma. However, some studies showed cases where the mutational status evaluated in the plasma did not totally represent the mutational status in the tumour [108]. Tissue is still the choice when it possible to biopsy. In negative cases, it is necessary to recur to tissue and to apply more sensitive methods. Tissue biopsy is still the gold standard, but liquid biopsy could be adequate specially to overcome the problems related to biopsies.

Genetic/molecular heterogeneity is recognized when we consider the most frequent mutations according to the histologic type. *EGFR*, *KRAS*, *P53* mutations and *ALK*, *RET*, *ROS1* rearrangements and *EGFR* and *MET* amplifications are more frequently identified in lung adenocarcinomas [77, 140–146]. P53, PI3KCA mutations and FGFR1 amplification are more frequently identified in lung squamous cell carcinomas [77, 145, 147–150]. RB and P53 mutations and MYC amplification are more frequently identified in small cell lung cancer [42, 77, 141, 151].

Our studies in bronchial-pulmonary carcinomas, combining immunohistochemical and molecular pathology testing, have demonstrated the following cascades in pulmonary carcinomas: epidermoid carcinoma – EGFR and HER2 polysomy and CK7/Vimentin for EMT non-pure epidermoid carcinomas; bronchial-pulmonary adenocarcinomas – non-smoking females – mutated EGFR and ERCC1 expression; micropapillary pattern with VIM/RB/ERCC1 expression; acinar/BA-lepidic/micropapillary patterns express TTF1 and mutated EGFR [18, 47, 152].

We verified that generally EGFR mutations were present in all the patterns of the same adenocarcinoma, reinforcing the possibility of mutational status determination in biopsies [47]. Some other authors also identified that the identification of EGFR mutations was independent of the localizations in the primary tumour and concordant with metastasis [80]. However, we did find some cases where the mutations were not present in all the patterns [47]. We also found cases with harbouring different EGFR mutations in different patterns, nevertheless all mutations were activating mutations [47]. We have also identified cases with more than one type of EGFR mutation in the same patterns/cells of the same pattern and even have published cases with EGFR and KRAS mutation coexistence [47]. These facts are in favour of the tumour heterogeneity hypothesis as these complex EGFR mutations (coexistence of more than one type) were detected in adenocarcinomas, clearly demonstrating a molecular complexity that might be related to different cell clones or genomic instability responsible for the accumulation of multiple molecular events in the EGFR gene.

Our group has also investigated DNA promoter hypermethylation according to histologic type. We also found heterogeneity when studying MHL1 and MSH2 gene methylation [153]. A higher prevalence of the MLH1 gene was identified mainly in squamous cell carcinoma (72%) [153]. However, no obvious differences were found for MSH2 promoter hypermethylation [153].

FGFR1 was recently considered as a driver oncogene, especially for squamous cell carcinoma [154–156]. Some authors have identified overexpression and increased gene copy number especially in squamous cell carcinomas, an example of inter-tumour heterogeneity.

In our research, we have evaluated the FGFR1 gene, not only in squamous cell carcinoma but also in other histological types. We found FGFR1 protein expression in all subtypes of lung bronchial-pulmonary carcinomas, especially in pleomorphic carcinomas [157]. FGFR1 amplification, although more frequent in squamous cell carcinoma, was also identified in adenocarcinomas, adenosquamous and pleomorphic carcinomas [157]. Higher expression in pleomorphic carcinomas suggests that overexpression may also be implicated in the activation of the EMT pathway [157]. Overexpression could also be responsible for tumour growth and proliferation and invasiveness, related with a more aggressive behaviour [157].

Inter-tumour heterogeneity can also be identified at a metabolic level as some works of our groups of research demonstrate [158–162]. Distinct metabolic signatures have been found between lung adenocarcinomas between adenocarcinomas and squamous cell carcinomas. These findings could be identified in several biological specimens as neoplastic tissue, plasma, and urine [158–162]. The authors argue that RMN-based technologies could be used for diagnostic perposes after validation [158–162].

The development of next-generation sequencing methods allowed to easily demonstrate intra-tumour and inter-tumour genetic heterogeneity.

Thus, it is very important to know that lung cancer heterogeneity is a fact, with implications in pathogenesis understanding, carcinogenesis, pathological diagnosis, selection of tissue for molecular diagnosis, and in therapeutic decision. The understanding of tumour heterogeneity is crucial and we must be aware of the implications and future developments regarding this field.

Tumour heterogeneity could be addressed by different perspectives; for the pathologist as histological or pattern differences in the tumour, for the molecular pathologist as genetic/molecular and epigenetic variations in the tumour (spatial and temporal), and for the oncologist as heterogeneity related to therapeutic response and resistance.

Tumour heterogeneity is changing the paradigm: initially one treatment was fitted to all patients, then, with the emergence of targeted therapies, one patient/one treatment was applied, and now we are heading towards precision medicine, i.e., one patient/one moment in the disease evolution/one treatment.

References

1 Sousa V, Espirito Santo J, Silva M, Cabral T, Alarcao AM, et al: EGFR/erB-1, HER2/erB-2, CK7, LP34, Ki67 and P53 expression in preneoplastic lesions of bronchial epithelium: an immunohistochemical and genetic study. Virchows Arch 2011;458:571–581.

2 Franklin WA, Veve R, Hirsch FR, Helfrich BA, Bunn PA Jr: Epidermal growth factor receptor family in lung cancer and premalignancy. Semin Oncol 2002;29:3–14.

3 Herbst RS, Bunn PA Jr: Targeting the epidermal growth factor receptor in non-small cell lung cancer. Clin Cancer Res 2003;9:5813–5824.

4 Kurie JM, Shin HJ, Lee JS, Morice RC, Ro JY, et al: Increased epidermal growth factor receptor expression in metaplastic bronchial epithelium. Clin Cancer Res 1996;2:1787–1793.

5 Lonardo F, Dragnev KH, Freemantle SJ, Ma Y, Memoli N, et al: Evidence for the epidermal growth factor receptor as a target for lung cancer prevention. Clin Cancer Res 2002;8: 54–60.

6 Meert AP, Martin B, Delmotte P, Berghmans T, Lafitte JJ, et al: The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. Eur Respir J 2002;20:975–981.

7 Meert AP, Verdebout JM, Martin B, Ninane V, Feoli F, et al: Epidermal growth factor receptor expression in pre-invasive and early invasive bronchial lesions. Eur Respir J 2003; 21:611–615.

8 Piyathilake CJ, Frost AR, Manne U, Weiss H, Bell WC, et al: Differential expression of growth factors in squamous cell carcinoma and precancerous lesions of the lung. Clin Cancer Res 2002;8:734–744.

9 Rusch V, Baselga J, Cordon-Cardo C, Orazem J, Zaman M, et al: Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res 1993;53:2379–2385.

10 Giatromanolaki A, Koukourakis MI, O'Byrne K, Kaklamanis L, Dicoglou C, et al: Non-small cell lung cancer: c-erbB-2 overexpression correlates with low angiogenesis and poor prognosis. Anticancer Res 1996;16:3819–3825.

11 Hirsch FR, Scagliotti GV, Langer CJ, Varella-Garcia M, Franklin WA: Epidermal growth factor family of receptors in preneoplasia and lung cancer: perspectives for targeted therapies. Lung Cancer 2003;41(suppl 1):S29–S42.

12 Betticher DC, Heighway J, Thatcher N, Hasleton PS: Abnormal expression of CCND1 and RB1 in resection margin epithelia of lung cancer patients. Br J Cancer 1997;75:1761–1768.

13 Brambilla E, Gazzeri S, Lantuejoul S, Coll JL, Moro D, et al: p53 mutant immunophenotype and deregulation of p53 transcription pathway (Bcl2, Bax, and Waf1) in precursor bronchial lesions of lung cancer. Clin Cancer Res 1998;4:1609–1618.

14 Hirano T, Franzen B, Kato H, Ebihara Y, Auer G: Genesis of squamous cell lung carcinoma. Sequential changes of proliferation, DNA ploidy, and p53 expression. Am J Pathol 1994; 144:296–302.

15 Li ZH, Zheng J, Weiss LM, Shibata D: c-k-ras and p53 mutations occur very early in adenocarcinoma of the lung. Am J Pathol 1994;144: 303–309.

16 Meert AP, Martin B, Verdebout JM, Feoli F, Mascaux C, et al: EGFR, c-erbB-2 and ki-67 in NSCLC and preneoplastic bronchial lesions. Anticancer Res 2006;26:135–138.

17 Satoh Y, Ishikawa Y, Nakagawa K, Hirano T, Tsuchiya E: A follow-up study of progression from dysplasia to squamous cell carcinoma with immunohistochemical examination of p53 protein overexpression in the bronchi of ex-chromate workers. Br J Cancer 1997;75: 678–683.

18 Sousa V, Bastos B, Silva M, Alarcao AM, Carvalho L: Bronchial-pulmonary adenocarcinoma subtyping relates with different molecular pathways. Rev Port Pneumol 2006;21:259–270.
19 Tanaka H, Yanagisawa K, Shinjo K, Taguchi A, Maeno K, et al: Lineage-specific dependency of lung adenocarcinomas on the lung development regulator TTF-1. Cancer Res 2007;67:6007–6011.
20 Sumiyoshi S, Yoshizawa A, Sonobe M, Kobayashi M, Sato M, et al: Non-terminal respiratory unit type lung adenocarcinoma has three distinct subtypes and is associated with poor prognosis. Lung Cancer 2014;84:281–288.
21 Myong NH: Thyroid transcription factor-1 (TTF-1) expression in human lung carcinomas: its prognostic implication and relationship with expressions of p53 and Ki-67 proteins. J Korean Med Sci 2003;18:494–500.
22 Perner S, Wagner PL, Soltermann A, LaFargue C, Tischler V, et al: TTF1 expression in non-small cell lung carcinoma: association with TTF1 gene amplification and improved survival. J Pathol 2009;217:65–72.
23 Berghmans T, Paesmans M, Mascaux C, Martin B, Meert AP, et al: Thyroid transcription factor 1 – a new prognostic factor in lung cancer: a meta-analysis. Ann Oncol 2006;17:1673–1676.
24 Haque AK, Syed S, Lele SM, Freeman DH, Adegboyega PA: Immunohistochemical study of thyroid transcription factor-1 and HER2/neu in non-small cell lung cancer: strong thyroid transcription factor-1 expression predicts better survival. Appl Immunohistochem Mol Morphol 2002;10:103–109.
25 Tan D, Li Q, Deeb G, Ramnath N, Slocum HK, et al: Thyroid transcription factor-1 expression prevalence and its clinical implications in non-small cell lung cancer: a high-throughput tissue microarray and immunohistochemistry study. Hum Pathol 2003;34:597–604.
26 Barlesi F, Pinot D, Legoffic A, Doddoli C, Chetaille B, et al: Positive thyroid transcription factor 1 staining strongly correlates with survival of patients with adenocarcinoma of the lung. Br J Cancer 2005;93:450–452.
27 Pelosi G, Fraggetta F, Pasini F, Maisonneuve P, Sonzogni A, et al: Immunoreactivity for thyroid transcription factor-1 in stage I non-small cell carcinomas of the lung. Am J Surg Pathol 2001;25:363–372.
28 Shanzhi W, Yiping H, Ling H, Jianming Z, Qiang L: The relationship between TTF-1 expression and EGFR mutations in lung adenocarcinomas. PLoS One 2014;9:e95479.
29 Sun PL, Seol H, Lee HJ, Yoo SB, Kim H, et al: High incidence of EGFR mutations in Korean men smokers with no intratumoral heterogeneity of lung adenocarcinomas: correlation with histologic subtypes, EGFR/TTF-1 expressions, and clinical features. J Thorac Oncol 2012;7:323–330.
30 Yatabe Y, Kosaka T, Takahashi T, Mitsudomi T: EGFR mutation is specific for terminal respiratory unit type adenocarcinoma. Am J Surg Pathol 2005;29:633–639.
31 Yatabe Y: EGFR mutations and the terminal respiratory unit. Cancer Metastasis Rev 2010;29:23–36.
32 Giaccone G, Gallegos Ruiz M, Le Chevalier T, Thatcher N, Smit E, et al: Erlotinib for front-line treatment of advanced non-small cell lung cancer: a phase II study. Clin Cancer Res 2006;12:6049–6055.
33 Peterson MR, Piao Z, Bazhenova LA, Weidner N, Yi ES: Terminal respiratory unit type lung adenocarcinoma is associated with distinctive EGFR immunoreactivity and EGFR mutations. Appl Immunohistochem Mol Morphol 2007;15:242–247.
34 Hwang IG, Ahn MJ, Park BB, Ahn YC, Han J, et al: ERCC1 expression as a prognostic marker in N2(+) nonsmall-cell lung cancer patients treated with platinum-based neoadjuvant concurrent chemoradiotherapy. Cancer 2008;113:1379–1386.
35 Rosell R, Lord RV, Taron M, Reguart N: DNA repair and cisplatin resistance in non-small-cell lung cancer. Lung Cancer 2002;38:217–227.
36 Rosell R, Taron M, Barnadas A, Scagliotti G, Sarries C, et al: Nucleotide excision repair pathways involved in Cisplatin resistance in non-small-cell lung cancer. Cancer Control 2003;10:297–305.
37 Seve P, Dumontet C: Chemoresistance in non-small cell lung cancer. Curr Med Chem Anticancer Agents 2005;5:73–88.
38 Simon GR, Sharma S, Cantor A, Smith P, Bepler G: ERCC1 expression is a predictor of survival in resected patients with non-small cell lung cancer. Chest 2005;127:978–983.
39 Zhou W, Gurubhagavatula S, Liu G, Park S, Neuberg DS, et al: Excision repair cross-complementation group 1 polymorphism predicts overall survival in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. Clin Cancer Res 2004;10:4939–4943.
40 Russell PA, Wainer Z, Wright GM, Daniels M, Conron M, et al: Does lung adenocarcinoma subtype predict patient survival? A clinicopathologic study based on the new International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society international multidisciplinary lung adenocarcinoma classification. J Thorac Oncol 2011;6:1496–1504.
41 Russell PA, Wright GM: Predominant histologic subtype in lung adenocarcinoma predicts benefit from adjuvant chemotherapy in completely resected patients: discovery of a holy grail? Ann Transl Med 2016;4:16.
42 Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, et al: International association for the study of lung cancer/American Thoracic Society/European Respiratory Society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 2011;6:244–285.
43 Yoshizawa A, Motoi N, Riely GJ, Sima CS, Gerald WL, et al: Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. Mod Pathol 2011;24:653–664.
44 Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Fujimoto M, et al: Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. J Thorac Oncol 2013;8:52–61.
45 Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG: WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon, IARC, 2015, p 412.
46 Song Z, Zhu H, Guo Z, Wu W, Sun W, et al: Correlation of EGFR mutation and predominant histologic subtype according to the new lung adenocarcinoma classification in Chinese patients. Med Oncol 2013;30:645.
47 Sousa V, Rodrigues C, Silva M, Alarcao AM, Carvalho L: Lung adenocarcinoma: sustained subtyping with immunohistochemistry and EGFR, HER2 and KRAS mutational status. Rev Port Pneumol 2006;21:113–125.
48 Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, et al: Diagnosis of lung adenocarcinoma in resected specimens: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. Arch Pathol Lab Med 2013;137:685–705.
49 Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, et al: International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society: international multidisciplinary classification of lung adenocarcinoma: executive summary. Proc Am Thorac Soc 2011;8:381–385.
50 Travis WD, Brambilla E, Riely GJ: New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. J Clin Oncol 2013;31:992–1001.
51 Wright GM, Do H, Weiss J, Alam NZ, Rathi V, et al: Mapping of actionable mutations to histological subtype domains in lung adenocarcinoma: implications for precision medicine. Oncotarget 2014;5:2107–2115.
52 Tochigi N, Dacic S, Nikiforova M, Cieply KM, Yousem SA: Adenosquamous carcinoma of the lung: a microdissection study of KRAS and EGFR mutational and amplification status in a western patient population. Am J Clin Pathol 2011;135:783–789.
53 Pelosi G, Scarpa A, Manzotti M, Veronesi G, Spaggiari L, et al: K-ras gene mutational analysis supports a monoclonal origin of biphasic pleomorphic carcinoma of the lung. Mod Pathol 2004;17:538–546.
54 Fang B, Mehran RJ, Heymach JV, Swisher SG: Predictive biomarkers in precision medicine and drug development against lung cancer. Chin J Cancer 2015;34:295–309.

55 Attard G, Jameson C, Moreira J, Flohr P, Parker C, et al: Hormone-sensitive prostate cancer: a case of ETS gene fusion heterogeneity. J Clin Pathol 2009;62:373–376.
56 Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, et al: The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature 2010;467:1109–1113.
57 Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, et al: Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet 2014;46:225–233.
58 Fidler IJ: Tumor heterogeneity and the biology of cancer invasion and metastasis. Cancer Res 1978;38:2651–2660.
59 Marjanovic ND, Weinberg RA, Chaffer CL: Poised with purpose: cell plasticity enhances tumorigenicity. Cell Cycle 2013;12:2713–2714.
60 Marjanovic ND, Weinberg RA, Chaffer CL: Cell plasticity and heterogeneity in cancer. Clin Chem 2013;59:168–179.
61 Marusyk A, Polyak K: Tumor heterogeneity: causes and consequences. Biochim Biophys Acta 2010;1805:105–117.
62 Marusyk A, Polyak K: Cancer. Cancer cell phenotypes, in fifty shades of grey. Science 2013;339:528–529.
63 Shackleton M, Quintana E, Fearon ER, Morrison SJ: Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell 2009;138:822–829.
64 Junttila MR, de Sauvage FJ: Influence of tumour micro-environment heterogeneity on therapeutic response. Nature 2013;501:346–354.
65 Kreso A, O'Brien CA, van Galen P, Gan OI, Notta F, et al: Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. Science 2013;339:543–548.
66 Cheng X, Chen H: Tumor heterogeneity and resistance to EGFR-targeted therapy in advanced nonsmall cell lung cancer: challenges and perspectives. Onco Targets Ther 2014;7:1689–1704.
67 Neelakantan D, Drasin DJ, Ford HL: Intratumoral heterogeneity: clonal cooperation in epithelial-to-mesenchymal transition and metastasis. Cell Adh Migr 2015;9:265–276.
68 Bose D, Zimmerman LJ, Pierobon M, Petricoin E, Tozzi F, et al: Chemoresistant colorectal cancer cells and cancer stem cells mediate growth and survival of bystander cells. Br J Cancer 2011;105:1759–1767.
69 Marusyk A, Almendro V, Polyak K: Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer 2012;12:323–334.
70 Aslakson CJ, Miller FR: Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. Cancer Res 1992;52:1399–1405.
71 Baban D, Matsumura Y, Kocialkowski S, Tarin D: Studies on relationships between metastatic and non-metastatic tumor cell populations using lineages labeled with dominant selectable genetic markers. Int J Dev Biol 1993;37:237–243.
72 Calbo J, van Montfort E, Proost N, van Drunen E, Beverloo HB, et al: A functional role for tumor cell heterogeneity in a mouse model of small cell lung cancer. Cancer Cell 2011;19:244–256.
73 Miller BE, Miller FR, Leith J, Heppner GH: Growth interaction in vivo between tumor subpopulations derived from a single mouse mammary tumor. Cancer Res 1980;40:3977–3981.
74 Miller BE, Miller FR, Wilburn D, Heppner GH: Dominance of a tumor subpopulation line in mixed heterogeneous mouse mammary tumors. Cancer Res 1988;48:5747–5753.
75 Tomonaga N, Nakamura Y, Yamaguchi H, Ikeda T, Mizoguchi K, et al: Analysis of intratumor heterogeneity of EGFR mutations in mixed type lung adenocarcinoma. Clin Lung Cancer 2013;14:521–526.
76 Bai H, Wang Z, Wang Y, Zhuo M, Zhou Q, et al: Detection and clinical significance of intratumoral EGFR mutational heterogeneity in Chinese patients with advanced non-small cell lung cancer. PLoS One 2013;8:e54170.
77 Sato MS, Shames DS, Girard L, Gazdar AF, Minna JD: Molecular basis of lung cancer; in Meloni D (ed): The Molecular Basis of Cancer, ed 3. Philadelphia, Saunders, 2008, pp 397–407.
78 D'Andrea AD: DNA repair pathways and human cancer; in Meloni D (ed): The Molecular Basis of Cancer, ed 3. Philadelphia, Saunders, 2008, pp 39–55.
79 Zhong WZ, Su J, Xu FP, Zhai HR, Zhang XC, et al: Rare discrepancies in a driver gene alteration within histologically heterogeneous primary lung cancers. Lung Cancer 2015;90:205–211.
80 Mansuet-Lupo A, Zouiti F, Alifano M, Tallet A, Charpentier MC, et al: Intratumoral distribution of EGFR mutations and copy number in metastatic lung cancer, what impact on the initial molecular diagnosis? J Transl Med 2014;12:131.
81 Kim HK, Lee HY, Choi YL, et al: Assessment of intratumoral heterogeneity of oncogenic driver mutations in surgically-resected lung adenocarcinoma: implications of percutaneous biopsy-based molecular assay for target-directed therapy. Anticancer Res 2014;34:707–714.
82 Taniguchi K, Okami J, Kodama K, Higashiyama M, Kato K: Intratumor heterogeneity of epidermal growth factor receptor mutations in lung cancer and its correlation to the response to gefitinib. Cancer Sci 2008;99:929–935.
83 Zhang J, Fujimoto J, Zhang J, Wedge DC, Song X, et al: Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. Science 2014;346:256–259.
84 Yatabe Y, Matsuo K, Mitsudomi T: Heterogeneous distribution of EGFR mutations is extremely rare in lung adenocarcinoma. J Clin Oncol 2011;29:2972–2977.
85 Mattsson JS, Imgenberg-Kreuz J, Edlund K, Botling J, Micke P: Consistent mutation status within histologically heterogeneous lung cancer lesions. Histopathology 2012;61:744–748.
86 Zhong WZ, Wu YL, Yang XN, Guo AL, Su J, et al: Genetic evolution of epidermal growth factor receptor in adenocarcinoma with a bronchioloalveolar carcinoma component. Clin Lung Cancer 2010;11:160–168.
87 Chen ZY, Zhong WZ, Zhang XC, Su J, Yang XN, et al: EGFR mutation heterogeneity and the mixed response to EGFR tyrosine kinase inhibitors of lung adenocarcinomas. Oncologist 2012;17:978–985.
88 Zito Marino F, Liguori G, Aquino G, La Mantia E, Bosari S, et al: Intratumor heterogeneity of ALK-rearrangements and homogeneity of EGFR-mutations in mixed lung adenocarcinoma. PLoS One 2015;10:e0139264.
89 Badalian G, Barbai T, Raso E, Derecskei K, Szendroi M, et al: Phenotype of bone metastases of non-small cell lung cancer: epidermal growth factor receptor expression and K-RAS mutational status. Pathol Oncol Res 2007;13:99–104.
90 Cortot AB, Italiano A, Burel-Vandenbos F, Martel-Planche G, Hainaut P: KRAS mutation status in primary nonsmall cell lung cancer and matched metastases. Cancer 2010;116:2682–2687.
91 Kalikaki A, Koutsopoulos A, Trypaki M, Souglakos J, Stathopoulos E, et al: Comparison of EGFR and K-RAS gene status between primary tumours and corresponding metastases in NSCLC. Br J Cancer 2008;99:923–929.
92 Schmid K, Oehl N, Wrba F, Pirker R, Pirker C, et al: EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. Clin Cancer Res 2009;15:4554–4560.
93 Sun L, Zhang Q, Luan H, Zhan Z, Wang C, et al: Comparison of KRAS and EGFR gene status between primary non-small cell lung cancer and local lymph node metastases: implications for clinical practice. J Exp Clin Cancer Res 2011;30:30.
94 Alsdorf WH, Clauditz TS, Hoenig T, Quaas A, Sirma H, et al: Intratumoral heterogeneity of KRAS mutation is rare in non-small-cell lung cancer. Exp Mol Pathol 2013;94:155–159.
95 Casadevall D, Gimeno J, Clave S, Taus A, Pijuan L, et al: MET expression and copy number heterogeneity in nonsquamous non-small cell lung cancer (nsNSCLC). Oncotarget 2015;6:16215–16226.
96 Remon J, Majem M: EGFR mutation heterogeneity and mixed response to EGFR tyrosine kinase inhibitors of non small cell lung cancer: a clue to overcoming resistance. Transl Lung Cancer Res 2013;2:445–448.
97 Kim EY, Cho EN, Park HS, Kim A, Hong JY, et al: Genetic heterogeneity of actionable genes between primary and metastatic tumor in lung adenocarcinoma. BMC Cancer 2016;16:27.

98 Sherwood J, Dearden S, Ratcliffe M, Walker J: Mutation status concordance between primary lesions and metastatic sites of advanced non-small-cell lung cancer and the impact of mutation testing methodologies: a literature review. J Exp Clin Cancer Res 2015;34:92.
99 Gow CH, Chang YL, Hsu YC, Tsai MF, Wu CT, et al: Comparison of epidermal growth factor receptor mutations between primary and corresponding metastatic tumors in tyrosine kinase inhibitor-naive non-small-cell lung cancer. Ann Oncol 2009;20:696–702.
100 Han C, Zou H, Ma J, Zhou Y, Zhao J: Comparison of EGFR and KRAS status between primary non-small cell lung cancer and corresponding metastases: a systematic review and meta-analysis. Zhongguo Fei Ai Za Zhi 2010;13:882–891.
101 Luo D, Ye X, Hu Z, Peng K, Song Y, et al: EGFR mutation status and its impact on survival of Chinese non-small cell lung cancer patients with brain metastases. Tumour Biol 2014;35:2437–2444.
102 Matsumoto S, Takahashi K, Iwakawa R, Matsuno Y, Nakanishi Y, et al: Frequent EGFR mutations in brain metastases of lung adenocarcinoma. Int J Cancer 2006;119: 1491–1494.
103 Park S, Holmes-Tisch AJ, Cho EY, Shim YM, Kim J, et al: Discordance of molecular biomarkers associated with epidermal growth factor receptor pathway between primary tumors and lymph node metastasis in non-small cell lung cancer. J Thorac Oncol 2009;4:809–815.
104 Shimizu K, Yukawa T, Hirami Y, Okita R, Saisho S, et al: Heterogeneity of the EGFR mutation status between the primary tumor and metastatic lymph node and the sensitivity to EGFR tyrosine kinase inhibitor in non-small cell lung cancer. Target Oncol 2013;8: 237–242.
105 Wei B, Yang K, Zhao J, Chang Y, Ma Z, et al: Quantification of EGFR mutations in primary and metastatic tumors in non-small cell lung cancer. J Exp Clin Cancer Res 2014; 33:5.
106 Li S, Rosell R, Urban A, Font A, Ariza A, et al: K-ras gene point mutation: a stable tumor marker in non-small cell lung carcinoma. Lung Cancer 1994;11:19–27.
107 Renovanz M, Kim EL: Intratumoral heterogeneity, its contribution to therapy resistance and methodological caveats to assessment. Front Oncol 2014;4:142.
108 Luo YH, Chen YM: Influence of chemotherapy on EGFR mutation status. Transl Lung Cancer Res 2013;2:442–444.
109 Bai H, Wang Z, Chen K, Zhao J, Lee JJ, et al: Influence of chemotherapy on EGFR mutation status among patients with non-small-cell lung cancer. J Clin Oncol 2012;30:3077–3083.
110 Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, et al: Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005;366:1527–1537.
111 Sharma SV, Bell DW, Settleman J, Haber DA: Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer 2007; 7:169–181.
112 Metz S, Ganter C, Lorenzen S, van Marwick S, Holzapfel K, et al: Multiparametric MR and PET imaging of intratumoral biological heterogeneity in patients with metastatic lung cancer using voxel-by-voxel analysis. PLoS One 2015;10:e0132386.
113 Kim DH, Jung JH, Son SH, Kim CY, Hong CM, et al: Prognostic significance of intratumoral metabolic heterogeneity on 18F-FDG PET/CT in pathological N0 non-small cell lung cancer. Clin Nucl Med 2015;40:708–714.
114 Hatt M, Majdoub M, Vallieres M, Tixier F, Le Rest CC, et al: 18F-FDG PET uptake characterization through texture analysis: investigating the complementary nature of heterogeneity and functional tumor volume in a multi-cancer site patient cohort. J Nucl Med 2015;56:38–44.
115 Kang SR, Song HC, Byun BH, Oh JR, Kim HS, et al: Intratumoral metabolic heterogeneity for prediction of disease progression after concurrent chemoradiotherapy in patients with inoperable stage III non-small-cell lung cancer. Nucl Med Mol Imaging 2014;48:16–25.
116 Munfus-McCray D, Cui M, Zhang Z, Gabrielson E, Askin F, et al: Comparison of EGFR and KRAS mutations in primary and unpaired metastatic lung adenocarcinoma with potential chemotherapy effect. Hum Pathol 2013;44:1286–1292.
117 Monaco SE, Nikiforova MN, Cieply K, Teot LA, Khalbuss WE, et al: A comparison of EGFR and KRAS status in primary lung carcinoma and matched metastases. Hum Pathol 2010;41:94–102.
118 Han HS, Eom DW, Kim JH, Kim KH, Shin HM, et al: EGFR mutation status in primary lung adenocarcinomas and corresponding metastatic lesions: discordance in pleural metastases. Clin Lung Cancer 2011;12:380–386.
119 Wu JY, Yu CJ, Shih JY, Yang CH, Yang PC: Influence of first-line chemotherapy and EGFR mutations on second-line gefitinib in advanced non-small cell lung cancer. Lung Cancer 2010;67:348–354.
120 Turtoi A, Blomme A, Castronovo V: Intratumoral heterogeneity and consequences for targeted therapies. Bull Cancer 2015;102: 17–23.
121 Roukos D, Batsis C, Baltogiannis G: Assessing tumor heterogeneity and emergence mutations using next-generation sequencing for overcoming cancer drugs resistance. Expert Rev Anticancer Ther 2012;12:1245–1248.
122 Lianos GD, Mangano A, Rausei S, Katsios C, Roukos DH: Tumor heterogeneity-based resistance guides personalized cancer medicine. Future Oncol 2014;10:1889–1892.
123 Francis G, Stein S: Circulating cell-free tumour DNA in the management of cancer. Int J Mol Sci 2015;16:14122–14142.
124 Huang WL, Wei F, Wong DT, Lin CC, Su WC: The emergent landscape of detecting EGFR mutations using circulating tumor DNA in lung cancer. Biomed Res Int 2015; 2015:340732.
125 Mateo J, Gerlinger M, Rodrigues DN, de Bono JS: The promise of circulating tumor cell analysis in cancer management. Genome Biol 2014;15:448.
126 Bordi P, Del Re M, Danesi R, Tiseo M: Circulating DNA in diagnosis and monitoring EGFR gene mutations in advanced non-small cell lung cancer. Transl Lung Cancer Res 2015;4:584–597.
127 Marchetti A, Palma JF, Felicioni L, De Pas TM, Chiari R, et al: Early prediction of response to tyrosine kinase inhibitors by quantification of EGFR mutations in plasma of NSCLC patients. J Thorac Oncol 2015;10: 1437–1443.
128 Marcq M, Vallee A, Bizieux A, Denis MG: Detection of EGFR mutations in the plasma of patients with lung adenocarcinoma for real-time monitoring of therapeutic response to tyrosine kinase inhibitors? J Thorac Oncol 2014;9:e49–e50.
129 Murtaza M, Dawson SJ, Tsui DW, Gale D, Forshew T, et al: Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 2013;497: 108–112.
130 Nakamura T, Sueoka-Aragane N, Iwanaga K, Sato A, Komiya K, et al: A noninvasive system for monitoring resistance to epidermal growth factor receptor tyrosine kinase inhibitors with plasma DNA. J Thorac Oncol 2011;6:1639–1648.
131 Oxnard GR, Paweletz CP, Kuang Y, Mach SL, O'Connell A, et al: Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. Clin Cancer Res 2014;20:1698–1705.
132 Piotrowska Z, Niederst MJ, Karlovich CA, Wakelee HA, Neal JW, et al: Heterogeneity underlies the emergence of EGFRT790 wild-type clones following treatment of T790M-positive cancers with a third-generation EGFR inhibitor. Cancer Discov 2015;5:713–722.
133 Sorensen BS, Wu L, Wei W, Tsai J, Weber B, et al: Monitoring of epidermal growth factor receptor tyrosine kinase inhibitor-sensitizing and resistance mutations in the plasma DNA of patients with advanced non-small cell lung cancer during treatment with erlotinib. Cancer 2014;120:3896–3901.
134 Thress KS, Paweletz CP, Felip E, Cho BC, Stetson D, et al: Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. Nat Med 2015;21:560–562.

135 Wang Y, Waters J, Leung ML, Unruh A, Roh W, et al: Clonal evolution in breast cancer revealed by single nucleus genome sequencing. Nature 2014;512:155–160.
136 Weber B, Meldgaard P, Hager H, Wu L, Wei W, et al: Detection of EGFR mutations in plasma and biopsies from non-small cell lung cancer patients by allele-specific PCR assays. BMC Cancer 2014;14:294.
137 Douillard JY, Ostoros G, Cobo M, Ciuleanu T, Cole R, et al: Gefitinib treatment in EGFR mutated Caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. J Thorac Oncol 2014;9: 1345–1353.
138 Mok T, Wu YL, Lee JS, Yu CJ, Sriuranpong V, et al: Detection and dynamic changes of EGFR mutations from circulating tumor DNA as a predictor of survival outcomes in NSCLC patients treated with first-line intercalated erlotinib and chemotherapy. Clin Cancer Res 2015;21:3196–3203.
139 Kim ST, Sung JS, Jo UH, Park KH, Shin SW, et al: Can mutations of EGFR and KRAS in serum be predictive and prognostic markers in patients with advanced non-small cell lung cancer (NSCLC)? Med Oncol 2013;30: 328.
140 Jang TW, Oak CH, Chang HK, Suo SJ, Jung MH: EGFR and KRAS mutations in patients with adenocarcinoma of the lung. Korean J Intern Med 2009;24:48–54.
141 Travis WD, Brambilla E, Müller-Hermelink HK, Harris CC: Pathology Genetics of Tumours of the Lung, Pleura, Thymus and Heart. Lyon, IARC Press, 2004.
142 van Zandwijk N, Mathy A, Boerrigter L, Ruijter H, Tielen I, et al: EGFR and KRAS mutations as criteria for treatment with tyrosine kinase inhibitors: retro- and prospective observations in non-small-cell lung cancer. Ann Oncol 2007;18:99–103.
143 Zhu CQ, da Cunha Santos G, Ding K, Sakurada A, Cutz JC, et al: Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 2008;26:4268–4275.
144 Cappuzzo F, Janne PA, Skokan M, Finocchiaro G, Rossi E, et al: MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. Ann Oncol 2009;20:298–304.
145 Chen HJ, Mok TS, Chen ZH, Guo AL, Zhang XC, et al: Clinicopathologic and molecular features of epidermal growth factor receptor T790M mutation and c-MET amplification in tyrosine kinase inhibitor-resistant Chinese non-small cell lung cancer. Pathol Oncol Res 2009;15:651–658.
146 Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, et al: MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007; 316:1039–1043.
147 Behrens C, Lin HY, Lee JJ, Raso MG, Hong WK, et al: Immunohistochemical expression of basic fibroblast growth factor and fibroblast growth factor receptors 1 and 2 in the pathogenesis of lung cancer. Clin Cancer Res 2008;14:6014–6022.
148 Berger W, Setinek U, Mohr T, Kindas-Mugge I, Vetterlein M, et al: Evidence for a role of FGF-2 and FGF receptors in the proliferation of non-small cell lung cancer cells. Int J Cancer 1999;83:415–423.
149 Donnem T, Al-Shibli K, Al-Saad S, Busund LT, Bremnes RM: Prognostic impact of fibroblast growth factor 2 in non-small cell lung cancer: coexpression with VEGFR-3 and PDGF-B predicts poor survival. J Thorac Oncol 2009;4:578–585.
150 Volm M, Koomagi R, Mattern J, Stammler G: Prognostic value of basic fibroblast growth factor and its receptor (FGFR-1) in patients with non-small cell lung carcinomas. Eur J Cancer 1997;33:691–693.
151 Gray MJG, Gallick GE: The role of oncogene activation in tumor progression; in Coppola D (ed): Mechanisms of Oncogenesis: An Update on Tumorigenesis. Heidelberg, Springer, 2010, pp 19–41.
152 Couceiro P, Sousa V, Alarcao A, Silva M, Carvalho L: Polysomy and amplification of chromosome 7 defined for EGFR gene in squamous cell carcinoma of the lung together with exons 19 and 21 wild type. Rev Port Pneumol 2010;16:453–462.
153 Gomes A, Reis-Silva M, Alarcao A, Couceiro P, Sousa V, et al: Promoter hypermethylation of DNA repair genes MLH1 and MSH2 in adenocarcinomas and squamous cell carcinomas of the lung. Rev Port Pneumol 2014;20:20–30.
154 Dutt A, Ramos AH, Hammerman PS, Mermel C, Cho J, et al: Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS One 2011;6:e20351.
155 Weiss J, Sos ML, Seidel D, Peifer M, Zander T, et al: Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010;2: 62ra93.
156 Ware KE, Hinz TK, Kleczko E, Singleton KR, Marek LA, et al: A mechanism of resistance to gefitinib mediated by cellular reprogramming and the acquisition of an FGF2-FGFR1 autocrine growth loop. Oncogenesis 2013;2:e39.
157 Sousa V, Reis D, Silva M, Alarcao AM, Ladeirinha AF, et al: Amplification of FGFR1 gene and expression of FGFR1 protein is found in different histological types of lung carcinoma. Virchows Arch 2016;469:173–182.
158 Duarte IF, Rocha CM, Barros AS, Gil AM, Goodfellow BJ, et al: Can nuclear magnetic resonance (NMR) spectroscopy reveal different metabolic signatures for lung tumours? Virchows Arch 2010;457:715–725.
159 Rocha CM, Barros AS, Gil AM, Goodfellow BJ, Humpfer E, et al: Metabolic profiling of human lung cancer tissue by 1H high resolution magic angle spinning (HRMAS) NMR spectroscopy. J Proteome Res 2010;9:319–332.
160 Rocha CM, Barros AS, Goodfellow BJ, Carreira IM, Gomes A, et al: NMR metabolomics of human lung tumours reveals distinct metabolic signatures for adenocarcinoma and squamous cell carcinoma. Carcinogenesis 2015;36:68–75.
161 Rocha CM, Carrola J, Barros AS, Gil AM, Goodfellow BJ, et al: Metabolic signatures of lung cancer in biofluids: NMR-based metabonomics of blood plasma. J Proteome Res 2011;10:4314–4324.
162 Carrola J, Rocha CM, Barros AS, Gil AM, Goodfellow BJ, et al: Metabolic signatures of lung cancer in biofluids: NMR-based metabonomics of urine. J Proteome Res 2011;10: 221–230.