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SPECIAL ARTICLE

Chromosome 7 deregulation in non-small cell lung carcinoma molecular landscape

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Summary

Lung cancer exhibits an increasing incidence and a high mortality rate worldwide. Non-small cell lung carcinoma (NSCLC) constitutes the majority of patients with lung cancer (about 85% of all pathologically defined lung cancer cases). A broad spectrum of genomic imbalances, including chromosome polysomy/aneuploidy or specific gene deregulation mechanisms, such as point mutations, deletions and amplifications has been already identified in the corresponding patients, modifying their response rates to novel targeted therapeutic regimens, and affecting also their life span. Among all chromosomes, chromosome 7 seems to play a critical role in NSCLC development and progression. Aberrations in significant genes located on it, such as EGFR, cMET, BRAF combined with numerical abnormalities of the whole chromosome are cytogenetic events that lead to specific molecular signatures in patients with NSCLC. Detection of these chromosome/gene imbalances based on polymerase chain reaction (PCR) and in situ hybridization provides to oncologists the right genetic substrate for handling these patients in a rational therapeutic way regarding their isolated molecular profile. In the current paper, we present the structural and functional profile of chromosome 7 focused on its alterations in NSCLC.

Key words: *cancer, chromosome, gene, hybridization, lung*

Introduction

Cancer is a complicated disease involving a variety of gross chromosomal and specific gene alterations in its genesis, progression and metastatic expansion [1]. Concerning solid tumors, a variety of gene functional and numerical imbalances in crucial molecular pathways such as cell cycle regulation, signaling transduction, apoptosis or angiogenesis have been identified and explained [2]. Malignant cell transformation is mediated by an aberrant gene expression, including predominantly oncogenes' upregulation combined with suppressor genes' downregulation that lead to cell cycle deregulation [3]. In fact, cancer genome consists of all genetic alterations that modify the normal DNA/mRNA sequences, triggering thus a cataract of molecular reactions inside and outside the nucleus microenvironment [4]. Point mutations, polymorphisms, abnormal gene copy number (amplification, deletion), or structural chromosomal rearrangements (translocations) and epigenetic modifications detectable by different molecular techniques provide critical information to oncologists for handling these patients in a rational therapeutic way regarding

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their isolated molecular landscape [5].

Gross chromosome aberrations include abnormal numerical alterations such as polysomy – also aneuploidy – and monosomy detectable by karyotyping techniques and fluoresence *in situ* hybridization (FISH) analyses. Furthermore, structural changes and rearrangements (i.e. translocations) in specific or vast chromosome regions are identified by applying predominantly PCR and FISH, especially comparative genomic hybridization (CGH) [6,7]. In the current paper, we focused on chromosome 7 structural and functional abnormal aspects in NSCLC.

Introducing the chromosome 7: structure and genes

Based on a combination of FISH & PCR sequencing DNA and mRNA analyses, several study groups determined the DNA sequence of human chromosome 7, which was the first fully analyzed metacentric chromosome, representing approximately the 5.5% of the total DNA content in cells [8,9]. Inside its 99.4% of the well analyzed euchromatic sequence – comprising over 153 million base pairs – a number of 1,150 protein-coding genes and additionally 941 pseudogenes (DNA sequences similar to genes but without a functional role in producing proteins) has been already identified. Interestingly, human chromosome 7 was found to be genomically equal at 92% compared to the mouse analog [10]. Highly DNA conserved sequences in the two species were detected in many and critical for protein coding segments (26-46 at resolution of 330 to 100 kb, respectively). Inside its complete DNA sequence length, gene and exon coverages demonstrate 36.5 and 1.4% percentages, respectively, with a total gene density of 7.5% (per Mb). Chromosome 7 contains also many critical t-RNA genes (n=23) and human micro RNAs (mRNAs) [11].

Significant genes that lead to specific hereditary disorders are harbored on the long arm (7q) including cystic fibrosis, Williams-Beuren syndrome, Marfan & Ehlers-Danlos syndrome, glaucoma-related pigment dispersion syndrome, hereditary pancreatitis, Pendred syndrome, Zellweger syndrome, familial hypertrophic cardiomyopathy [12]. Similarly, Turcot syndrome, Charcot-Marie-Tooth neuropathy, macular dystrophy, Pallister-Hall syndrome, autosomal dominant mediated deafness, Shaethre-Chotzen syndrome, Stiff-Man syndrome and also familiar hyperinsulinism are important genes located on its short arm (7p) [13]. Besides these genes that are implicated in genetic inheriting and familial diseases, others are considered as carcinogenetic in solid and non-solid malignancies. Genes involved in the genesis of Ewing's sarcoma, hereditary non-polyposis colon cancer, T-cell tumor, glioblastoma, myeloid leukemia, Wilm's tumor are located on 7p arm, whereas papillary renal cell carcinoma (sporadic/familial), basal cell carcinoma, hepatocellular carcinoma (childhood variety) are referred to genes identified on 7q arm [14]. Furthermore, critical genes for signal transduction to the nucleus regulation are located on chromosome 7. Among them, epidermal growth factor receptor (EGFR-gene locus: 7p12, exons: 30), MET proto-oncogene, tyrosine kinase receptor (cMET-gene locus: 7q31, exons: 24), and also V-raf murine sarcoma viral oncogene homolog B1B-Raf proto-oncogene, serine/threonine kinase (BRAF- gene locus: 7q34, exons: 22) are frequently deregulated in solid malignancies including lung, colon, head & neck carcinomas [15-18]. Especially in NSCLC, these genes are correlated with established criteria for applying targeted therapeutic strategies in subgroups of patients characterized predominantly by specific mutations or amplification [19,20]. Ideogram of chromosome 7 and the corresponding genes are presented in Figure 1.

EGFR & chromosome 7 aberrations in NSCLC

EGFR protein acts as a transmembrane glycoprotein. It consists of a large extracellular ligand-binding region, a single hydrophobic transmembrane bridge adjusting to an intracellular juxtamembrane (JM) region, a tyrosine kinase

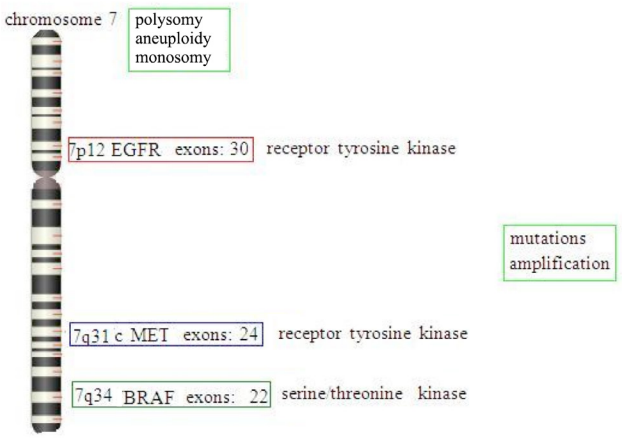

Figure 1. Ideogram of chromosome 7 focused on EGFR/c-MET/BRAF genes. Mechanisms of chromosome and gene deregulation in NSCLC are also presented.

domain and finally a C terminal tail with multiple tyrosine residues acting as a regulatory region [21]. Three main EGFR depended pathways have been already identified including the PI3K-AKT-PTEN-mTOR, the RAS-(B) RAF-MEK-ERK/MAPK and also the IL6-JAK1/2-STAT3 [22]. Concerning NSCLC, a subset of patients exhibits a specific genetic profile regarding EGFR activating mutations (approximately 10-30%). EGFR mutations (missense substitutions, in-frame insertions, in-frame deletions) modify the response rates (affordable response or activated resistance) to tyrosine kinase inhibitors (TKIs), such as erlotinib, gefitinib, afatinib, dacomitinib, vandetanib) and affect the survival of the corresponding patients [23]. Interestingly, T790M (exon 20) and L858R (exon 21) represent activating and also germline mutations, whereas V843I (exon 21) is a pure germline mutation [24]. Concerning EGFR numerical alterations detected by FISH in NSCLC, high gene copy numbers are found in almost 60% of the patients. Additionally, gene amplification leads to EGFR protein overexpression in the corresponding specimens (40-80%) detected by IHC. Although anti-EGFR monoclonal antibodies (mAbs, such as cetuximab, panitumumab) inhibition strategies in NSCLC patients are under consideration, gene amplification mechanism is the critical molecular event as it happens in HER2 gene amplified dependent breast cancers cases [25,26].

Chromosome 7 polysomy in EGFR amplified or non-amplified cases seems to play a crucial role in regulating EGFR protein expression levels. A balanced increase of EGFR gene and chromosome 7 copy numbers is related with specific EGFR mutations predicting also high probability of response to gefinitib [27]. Similarly, another study group explored the erlotinib efficacy in NSCLC patients with high polysomy of chromosome 7 and EGFR/KRas wild-type tumors. The authors concluded that high polysomy of chromosome 7 was the only molecular feature conferring clear signs of sensitivity to erlotinib [28]. Concerning the influence of chromosome 7 numerical imbalances in patients' survival rates, another recently published study showed that EGFR gene amplification combined with polysomy was found to be negatively correlated with poor differentiation and smoking history, combined with c-Kit and EGFR aberrant expression [29]. In contrast to chromosome 7 polysomy cases, the role of monosomy (loss of one chromosome inside the pair) in NSCLC is undetermined. A study group showed the experimentally loss of an EGFR-amplified chromosome 7 as a novel mechanism of acquired resistance to EGFR-TKIs in EGFR-mutated NSCLC cells, especially in low concentration of erlotinib [30].

c-MET & chromosome 7 aberrations in NSCLC

cMET proto-oncogene encodes hepatocyte growth factor tyrosine kinase receptor, which recognizes hepatocyte growth factor (HGF) as a ligand binding molecule. cMET regulates MEK/ERK, MEK/JNK and PI3K-Akt-antagonizing EGFR extracellular receptor activity- and indirectly MDM2 (mouse double minute 2) gene which controls p53 degradation [31]. cMET is involved also in IL6-JAK1/2-STAT3 by STAT3 phosporylation, which translocates to the nucleus acting as a transcription factor for several genes [32]. HGF/cMET protein overexpression due to gene amplification combined or not with chromosome 7 polysomy in NSCLC patients – mainly with adenocarcinoma harboring EGFR mutations (ie T790M) – acts as a mechanism of resistance in TKI application [33]. Anti-cMET targeted therapeutic strategies in these patients are under consideration. A study showed that by inhibiting cMET expression through shRNA the sensitivity to EGFR-TKIs was restored [34]. In contrast to these findings, there is a skepticism regarding strategies based on simultaneous EGFR/cMET (dual) inhibition, although the synergistic effect of specific inhibitors has been detected in *in vitro* experimental models [35]. Additionally to these molecular events, 7q31 region analysis by FISH should be used also as a reliable marker for chromosome 7 numerical imbalances determination. A study group investigated the usefulness of 7q31 region evaluation to discriminate EGFR amplification from chromosome 7 polysomy in controversial EGFR FISH positive cases. The authors concluded that simultaneous 7p12 & 7q31 FISH analysis provide a secure result regarding the identification of pure EGFR amplification combined with or not chromosome 7 polysomy/aneuploidy [36].

BRAF & chromosome 7 aberrations in NSCLC

The BRAF protein - encoded by the corresponding gene - belongs to the raf/mil family of serine/threonine protein kinases regulating the MAP kinase/ERKs signaling pathway [37]. Point mutations inside this gene's exons have been detected in NSCLC patients. The mutational rate is estimated between 1 to 4% in lung adenocarci-

noma cases [38]. BRAF V600E is a driving mutation that can be effectively targeted using selective BRAF and/or MEK inhibitors. There is also a significant number of other point mutations in different exons of the gene with an undetermined role in NSCLC response or resistance in TKIs (dabrafenib, vemurafenib) [39,40]. Based on FISH analyses, a study group detected BRAF gene amplification (predominantly low) in sporadic cases of NSCLC patients. Interestingly, BRAF V600E status was correlated with a BRAF increased copy number. Chromosome 7 polysomy was also identified focally. They suggested that the combination of BRAF copy number gain and V600E mutation may serve as a marker of the more aggressive biological behavior in lung adenocarcinoma due to increased N stage in TNM taxonomy (lymph node positive cases) [41].

Conclusions

NSCLC represents the vast majority of lung carcinomas exhibiting a broad spectrum of chromosome and specific gene rearrangements [42]. Intra-tumoral genomic heterogeneity modifies the response rates of the patients to targeted therapeutic strategies (TKIs or mAbs). Chromosome 7 deregulation due to aneuploidy/polysomy/monosomy or single/complex gene abnormalities (mutation/amplification) plays a crucial role in these malignancies increasing their aggressive biological behavior. A recently published study showed that glucococticoid receptor regulates mitotic progression and its reduced expression is detected in a panel of human liver, lung, prostate, colon and breast cancers [43]. Similar molecular analyses will improve our molecular knowledge in understanding mechanisms that induce chromosomal instability.

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