Extended Abstract Research & Reviews: Medical and Clinical Oncology 2019
Vol.3 No.1

Genetic Alterations and Tumor Mutation Burden of Poorly Differentiated Small Cell Euro-endocrine Carcinomas are Similar in Lung Lesions and Distant Metastatic Foci

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Study the genetic alterations of poorly differentiated small cell neuroendocrine carcinomas to improve understanding of the biology of these aggressive cancers.

Next generation sequencing was performed on DNA samples, using Illumina HiSeq2000 / 4000 on 315 cancer-related genes and a burden of tumor mutation has been reported: in 914 small cell lung cancers (SCLC) and 115 small undefined primary cells (SCUP), there were similar and close rates of genetic damage in pulmonary lesions and distant metastatic foci in SCLC and SCUP. In addition, the majority of tumors, both lung lesions and distant metastatic foci, did not carry a high tumor mutation load. Several potentially targetable pilot genes have been identified. Despite the common involvement of transmembrane signaling pathways and transcription mechanisms, other than TP53 and RB1, there has been no significant simultaneous genetic alteration.

Neuroendocrine tumors are a wide range of different neoplasms originating from endocrine origin and the nervous system. They generally express chromogranin A, synaptophysin (p38), the neuronal adhesion molecule (CD56), the neuron-specific enolase or the neurofilament. They are generally classified according to their degree of differentiation, their grade and their mitotic rate. Poorly differentiated small cell neuroendocrine carcinomas are small round blue cells with a rapid proliferation rate, lack of structural formation and poor differentiation. These cancers are aggressive, invade and metastasize early, respond to chemotherapy and radiation, and relapse almost everywhere. The most common type is small cell lung cancer (SCLC), which accounts for 15% of lung cancers with a higher incidence in older men with a history of smoking [1-6]. However, they can occur in many different organs and can also be present in other cancers. The presence of a component of the small cell neuroendocrine neoplasm in other tumors is associated with a poor prognosis [7-9]. Many previous studies on small carcinoma cell lines, xenograines and primary human tumors have reported a variety of mutations, most commonly TP53 and RB1 [10-12]. These studies have shown variable genetic mutations in tumors from different organs. For example, some have shown similar genetic damage in small cell cancers occurring in the pancreas and esophagus, but have shown different results in these rare cancers originating in the bladder [13-15]. A similar mutational analysis leads to the reclassification of small cell carcinoma of the ovaries as an ovarian rhabdoid tumor [16]. Similarly, other studies have identified the papillomavirus associated with Merkel cells as a cause of this poorly differentiated small cell skin cancer [17,18]. Understanding the genetic pathways modified in tumors can help to understand their biology, the mechanisms involved in their development, their appropriate classification and potentially help to identify therapeutic strategies. Previous studies with a smaller sample had shown limited co-occurrence of genetic damage and comparing genetic damage to metastatic lesions in different organs [10,19-21]. In order to improve our understanding of the genes and pathways involved in these tumors, the present study compares mutations in a broader set of lung lesions, distant metastatic foci and undefined primitive small cell carcinoma (SCUP), and also assesses the co-occurrence of these mutations. The methods of genetic sequencing are fully explained in a previous publication [22]. In short, samples were diagnosed by a local pathologist, then slides were submitted for full genomic profiling based on hybrid capture (CGP) at Foundation Medicine (Cambridge, MA). Samples of tumors analyzed between January 2010 and December 2016 were included. The origins of the primary tumor were based on the documented report of the requesting local institutions. Tumors without documented origin have been classified as undefined primitive small cell carcinoma (SCUP). DNA was extracted from formaldehyde-integrated biopsy or from surgical specimens. CGP was performed using Illumina HiSeq2000 / 4000 on adapter ligated, hybridization-capture indexing libraries for exons of 315 cancer-related genes and 47 introns of 19 genes frequently involved in Studying the genetic alterations of poorly differentiated small cell neuroendocrine carcinomas to improve the understanding of the biology of these aggressive cancers.

Next generation sequencing was performed on the DNA extracted samples, using the Illumina HiSeq2000/4000 on 315 cancer related genes and tumor mutation burden was reported.: In 914 small cell lung cancer (SCLC) and 115 small cell of undefined primary (SCUP), there were similar and close rates of genetic alterations in lung lesions and distant metastatic foci in SCLC and SCUP. Also, the majority of tumors, both lung lesions and distant metastatic foci, did not carry a high tumor mutation burden. Multiple potentially targetable driver genes were identified. Despite common involvement of transmembrane signaling pathways and transcription machinery, other than TP53 and RB1, there was no considerable concurrent gene alteration.

Neuroendocrine tumors are a wide array of di erent neoplasms arising from endocrine and nervous system origin. Hey commonly express chromogranin A, synaptophysin (p38), neural adhesion molecule (CD56), neuron-specific enolase, or neurofilament. Hey are generally categorized based on their degree of di erentiation, grade and mitotic rate. Small cell poorly di erentiated neuroendocrine carcinomas are small round blue cells with rapid proliferation rate, lack of structural

This work is partly presented at 15th Asia Pacific Oncologists Annual Meeting, September 05-06, 2018 Tokyo, Japan

formation, and poor differentiation. Hese cancers are aggressive, invade and metastasize early, respond to chemotherapy and radiation, and relapse almost universally. He most common type is the small cell lung cancer (SCLC), counting for 15% of lung cancers with higher incidence among older males with smoking history [1-6]. However, they can arise in many different organs and can be present in other cancers as well. Presence of a component of small cell neuroendocrine neoplasm in other tumors is associated with poor prognosis [7-9]. Multiple prior studies on small carcinoma cell lines, xenograis and primary human tumors have reported a variety of mutations, most commonly TP53 and RB1 [10-12]. Hese studies have shown variable genetic mutations in tumors arising from different organs. As an example, some have showed similar genetic alterations in small cell cancers arising in the pancreas and esophagus but showed different findings in those rare cancers originating in the bladder [13-15]. Similar mutational analysis leads to re-classification of small cell carcinoma of the ovaries as ovarian rhabdoid tumor [16]. Similarly, other studies have helped identify Merkel cell associate papilloma virus as a cause of this small cell poorly differentiated cancer of skin [17,18]. Understanding the altered genetic pathways in the tumors may help understand their biology, mechanisms involved in their development, their proper classification and potentially help with identifying therapeutic strategies. Previous studies with smaller sample size had shown limited co-occurrence of the genetic alterations and comparing genetic alterations in metastasis lesions in different organs [10,19-21]. In order to improve our understanding of the genes and pathways involved in these tumors, the current study compares mutations in a larger set of lung lesions, distant metastatic foci and small cell carcinoma of undefined primary (SCUP), and also evaluates cooccurrence of those mutations. Genetic sequencing methods are fully explained in a previous publication [22]. Briefly, samples were diagnosed by local pathologist and then slides were submitted for hybrid capture based comprehensive genomic profiling (CGP) to Foundation Medicine (Cambridge, MA). Tumor samples analyzed between January 2010 to December 2016 was included. Origins of the primary tumor were based on the documented report by the requesting local institutions. Tumors without documented origin were classified as small cell carcinoma of undefined primary (SCUP). DNA was extracted from formaldehyde fixed paraffin embedded biopsy or surgical specimens. CGP was performed using the Illumina HiSeq2000/4000 on indexed, adaptor ligated, hybridization-captures libraries for exons of 315 cancer related genes and 47 introns of 19 genes frequently involved in rearrangements. Genetic alterations included base substitutions, short insertions and deletions, amplifications, homologous deletions and chromosomal rearrangements. Alterations likely or known to be bona-fide oncogenic drivers and germ-line polymorphisms were included. Alterations were reported as short variants, copy number for genes (amplifications and losses), or rearrangements. Short variants include single-base nucleotide substitutions, small-scale multi-base deletions or insertions, and microsatellite repeats. Publicly available and validated analysis tools were used to analyze the data. Median exon unique coverage was 647X. For tumor mutation burden (TMB), the number of somatic mutations detected on NGS (interrogating 1.2 Mb of the genome) were quantified and that value extrapolated to the whole exome using a validated algorithm [23,24]. TMB was measured in mutations per mega base (Mb) and was divided into three groups: low (1-5 mutations/Mb), intermediate (6-19 mutations/Mb), and high (≥ 20 mutations/Mb). One hundred non-synonymous mutations per exome were used as a threshold. He threshold of 20 coding mutations per Mb was used as equivalent to 400 non-synonymous mutations per exome. In a large cohort of patients this approximately divided patients to 50% as low TMB, about 40% as intermediate TMB, and about 10% as high TMB [25]. Gene rearrangements were detected by identifying clusters of chimeric read pairs from both DNA (pairs mapping 0.10 kilo bases (kb) apart or on different chromosomes) and RNA (pairs mapping to RefSeq sequences corresponding to different genes or to genomic loci 0.10 kb apart). Chimera clusters were filtered for repetitive sequence and by distribution of mapped positions. Identified rearrangements were then annotated according to the genomic loci of both clusters and categorized as gene fusions, gene rearrangements, or truncatingevents. This study showed similar genetic alteration and tumor mutation burden in the lung lesions and in distant metastatic foci. TP53 and RB1 were the frequently altered concurrently.

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