Microsatellite instability detectable for Lung cancer Prognosis

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Abstract. Undoubtedly the accurate and fast diagnosis in early stages of lung cancer is very important to give patients a chance to give them the best treatment possible, but this is not an easy process. There are several reasons that increase complexity and difficulty when we need to consider the fitness of the patient which itself may effect of the diagnostic of disease and treatment, so may require a change to the course of treatment or diagnostic pathway. In this study we will mention some new and latest strategies for diagnosis, prevention and treatment such as molecular techniques by microsatellite markers and others to reduce the severity of the cancer and on the other hand increased treatment efficiency.

1. Introduction
Lung cancer is the leading cause of cancer-related deaths in the United States [1]. In 2002, it was estimated that there would be approximately 169,400 new cases and 154,900 deaths attributed to lung cancer [1]. The American Cancer Society estimated that lung cancer in the United States was the second most common malignancy in both males and females. In comparison to all cancer types, the incidences of lung cancer were only 14% and 12% of total estimated new cases in males and females respectively. However only approximately 13% of lung cancer patients survive more than 5 years. Thus, the mortality rate of lung cancer is the highest for both males and females annually. It was estimated that lung cancer might contribute to 31% and 25% of the total cancer-related deaths in the United States in 2002 for males and females respectively. Therefore, lung cancer is a serious threat to human kind. For clinical diagnosis and treatment, lung cancer is divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [2]. Approximately 75% of lung tumors are NSCLC [3]. NSCLC tend to metastasize later in development, while SCLC spread very early.

Lung cancer is the result of a variety of a multistage process involving alterations in different genes that are resulting of diverse pathways. According of WHO the genes which involving of the lung cancer disease include tumor suppressor genes (TSG) which are negative growth regulators, proto-oncogenes which are positive growth regulators and DNA repair genes and apoptotic control genes. The polymorphisms of genes when inherited usually notably the carcinogen metabolism genes develop the Lung carcinogenesis, like development of other cancers with different of environmental exposures. Genes involved in multiple pathway in cell cycle may be damaged at different stages of lung tumor progression. Many changes consist of alteration of chromosomal, copy number change, deregulated expression of telomerase and angiogenesis, microsatellite instability, lose of heterozygous. Mutational activation of oncogenes and inactivation of tumor suppressor genes, and subsequent increased genetic instability are major genetic events in lung carcinogens [4].
Genetic diagnosis of Lung cancer can mean at least three types which are different based on genetic methods an addition to hereditary cancer risk or to presymptomatic/ prenatal diagnosis, these three include 1- detection of cancer cells using DNA samples isolated from blood, stool, sputum, or urine. 2- genetic diagnostic of malignancy or bening status; 3- characterization of individual cancer cells to examine their spread, if the tumor is metastatic and whether the cells sensitive to anticancer drugs or irradiation.

Genomic testing can find DNA alterations that drives the growth of cancer. They can understand what caused the tumor and the treatment of it by finding and identifying the mutations which occur in a genome of the tumor cell, the genetic diagnostic able to provide cancer treatment therapies that specifically target changes in the profile of genomic of tumor cell and it is may helping to determine treatments that may be appropriate. It is important that the individual treatment of the patient is identical to his exact genetic diagnosis [5]. MSI can’t be the unique cause of lung cancer, given that there is a number of cases that do not presents any MSI [6]. This form of microsatellite instability (MSI) has become mark molecular signature of cancer, also DNA mismatch repair (MMR) genes responsible for cancers that lead to understand the cancer disease when genetic basis was identified and adoption to identifying and testing individuals at risk for cancer. This new milestone is important to early detection of cancer and identification of at-risk relatives is key to save lives, despite the challenges targeted immunotherapies offer new hope for treating the hereditary and sporadic of MSI-positive cancers [6]

1.1. Microsatellite

Microsatellite or SSRs (simple sequence repeats) consist of sequence of one to a few base that are repeated in tandem less than 10 to more than 100 times, it show high level of polymorphism. the widespread use of microsatellites, our understanding of their mutational behaviour, function, evolution and distribution in the genome and across taxa is increasing rapidly. It is varies in length between 5 and 40 repeats. Dinucleotide, trinucleotide and tetranucleotide repeats are the most common choices to using in for molecular studies. Many species as with dinucleotide repeats account for the majority of microsatellites, Trinucleotide and hexanucleotide repeats are the most likely repeat classes to appear in coding regions because they do not cause a frame shift, while the mononucleotide are less reliable because of problems with amplification; the less common are the longer repeat types [7]. Mechanism Microsatellite mutations are thought to arise from both a defective mismatch repair (MMR) enzyme system as well as replication error. More specifically, it is believed that micro satellite mutations arise from "DNA replication slippage". During DNA replication, the double helix unzips leading to the separation of the nascent and template DNA strands. DNA polymerase alpha then begins to synthesize a new strand of DNA along the template strand. On occasion, strands could reanneal out of register, white DNA polymerization continues to add one or more nucleotides to the previously replicated template strand. When DNA replication is complete, the template strand will correct itself by incorporating these short tandem repeated nucleotide sequences to match the newly synthesized DNA strand. This allows for a longer or shorter strand of DNA following replication, depending if an insertion or deletion occurred during replication[8][25]. The region of DNA where location around the microsatellite locus termed the flanking region so according this region can identified the variety between species or because the sequences of flanking regions are generally conserved across individuals of the same species and sometimes of different species, can be identified by application of real time PCR or classic PCR by designed oligonucleotides or primers to bind to the flanking region and guide the amplification of a microsatellite locus by this assay and use the microsatellite region as marker [7]. They can be distinguished by high resolution gel electrophoresis, which allows rapid genotyping of many individuals at many loci for a fraction of the price of sequencing DNA. Many microsatellites have high-mutation rates between 10-2 and 10-6 mutations per locus per generation, and on average 5 x 10-4 that generate the high levels of allelic diversity [9]. DNA replication microsatellite repeat sequences mutate frequently and the can change the number of repeats and the length of the DNA fragment are change so the can distinguished by different resolution in gel according the size of DNA fragments. Genomic microsatellite have been detected in the genomes of every organism analyzed so far [10]. Studies for lung cancer[2], were analyzed using a fairly small panel of microsatellite markers to identify allelic variation between the molecular signatures generated for cancer from an individual patient. The results presented in this study demonstrate that molecular analysis of allelic variations at polymorphic
microsatellite markers can be used to determine lineage relationships between multiple tumors, facilitating the discrimination of second primary cancer versus metastatic disease. This approach is a rapid and sensitive because the differ the sours of DNA samples were collected, so the results were differ if the sample obtained from tumor biopsy or from formalin-fixed paraffin-embedded. This method is potentially very useful in the molecular diagnostic of patients with multiple tumors [3]. Microsatellites are also outstanding markers for fluorescent techniques, high-throughput analyses and multiplexing [2].

1.2. Development of MSI markers

The MSI discovery lead to laboratories to progress their own methods for measuring MSI and started to test different types of cancers. There were no standards for MSI testing. Measurement criteria differed depending on the type and quantity of differences in microsatellite markers. Moreover, investigators differed on the percent of unstable markers necessary to classify a tumor as MSI-positive [5]. So it nearly impossible to compare results between laboratories and resulted in considerable variability in the frequency of MSI reported for a given tumor type. Because the disclear of standardization of assays for measuring MSI, some studies found that sensitivity and specificity of markers were closely related to the type of the repeat (highest for mono and dinucleotide repeats) and that MSI could be subdivided into MSI-H (>20% of markers were unstable), MSI-Low (MSI-L). Other studies test the reliability and quality of MSI analysis they suggested that the microsatellite panel should be comprised of different repeat types including mononucleotide and dinucleotide repeats. Cases were classified as MSI-positive and negative according the unstable markers [11].

Detection of MSI using of microsatellite markers

The recommendation of Bethesda guidelines in 2004 was said the use of a panel of all mononucleotide repeat markers to increase the sensitivity of detection of many types of cancer such as lung cancer. They suggested use the mono-markers more sensitivity to evaluate MSI, and to increase the sensitivity of detection [12]. The recommendation was mention MSI panel may underestimate the number of MSI-H tumors because of the use of dinucleotide repeats so suggested to be used more mononucleotide markers to evaluate MSI and improves the sensitivity [13]. Ninomiya and his colleagues the found the MSI method was important to the sensitivity of detection of lung cancer they concluded, the development of lung cancer depends on genomic instability at the chromosome level rather than at the nucleotide level. Microsatellite instability appears far less important in pathogenesis of lung cancer [12]. There were several studies conducted prior to the publication of the Bethesda Guidelines which established guidelines for the interpretation of MSI. Thibodeau et al. (1993) analyzed the DNA of colorectal tumors in order to look for somatic mutations in the form of (CA)n repeats, known as microsatellites. They found a series of genetic alterations that took the form of either amplifications or deletions in these repetitive DNA micro satellite sequences [26]. The frequency of MSI positive tumors varied widely from one paper to another, ranging from 0-33%. In fact, most of them tested a variety of different microsatellite markers, which created non-uniformity when analyzing for MSI. In addition, a variety of DNA extraction methods were utilized along with small variation in PCR technique [25].

1.3. Microsatellite alteration to distinguish metastatic of lung cancer

Among all other type of molecular markers microsatellite markers were the marker of choice to identify the diagnosis of many alteration and mutations in diseases, especially cancer including lung cancer where used. Many researchers have developed methods of genetic diagnosis using microsatellite markers. Tian, L and his colleagues developed a novel new molecular approach of comparative study for differentiation of tumor origin to evaluate the feasibility of distinguishing multiple primary lung cancer (MPLC) from metastatic lung cancer (MLC) through the analysis of microsatellite genomic instability of DNA. They concluded that identical microsatellite alteration could be observed in both circulating DNA and the tumor. With polymorphic microsatellite markers [14]. Many studies have demonstrated the possibility to detect genetic changes in DNA of cancer patients. Francesca ANDRIANI and his colleagues, validated of molecular approaches that might be useful for an effective early detection and monitoring of NSCLC. The goal of their study was to validate a panel of molecular markers for lung cancer detection in plasma DNA, by using microsatellite alterations at loci.
on chromosome 3. These results provide the proof of principle that plasma DNA alterations are tumor-specific in most cases and support blood testing as a noninvasive strategy for early detection[15].

1.4. Microsatellites markers and Next Generation Sequencing

There is no doubt that the Next Generation Sequencing (NGS) have provided a great service and a new scenario for the molecular diagnosis of many genetic diseases, especially cancer, as well as the detecting microsatellites of cancer. Where he provided several sequences available to the human genome and from them to generating an enormous quantity of made available in public databases and widely used for prospecting for microsatellites[16]. In the study by Bonneville et al [17], the investigators examined NGS data of the mismatch repair MMR genes and found MMR mutations in some cases of cancer, they provide evidence of as-yet-unappreciated MSI in several types of cancer. The study looked at mutational burden and found that MSI tumors have a significantly higher mutational burden compared with microsatellite stable (MSS) tumors. These findings support an expanded role for clinical MSI testing across multiple cancer types as patients with MSI-positive tumors are predicted to benefit from novel immunotherapies in clinical trials [17].

1.5. Advantages of microsatellite markers

Over the last few decades, the use of molecular markers has played an increasing role in molecular diagnostic. Of the different types of molecular markers, microsatellites have been utilized most extensively, because they can be readily amplified by PCR and the large amount of allelic variation at each locus [18]. In addition, microsatellite markers were used to diagnose cancer as explained above. Including lung cancer, the microsatellite marker assay has a lot of importance Advantages. Microsatellites are carry all characteristics that can be very suitable for the study of population structure, genealogy analysis and detection of differences between species and different disease conditions of cancer and Able to find the relationship between races and detecting differences among closely related species and biodiversity using pedigree analysis. Recently, attention has turned to another type of genetic variation that of differences in the number of repeated copies of a segment of DNA. These sequences can be classified based on decreasing sizes into satellites, minisatellites, and microsatellites [18]. By using microsatellites PCR assay can be identifying simple sequence repeat polymorphism of Lung cancer [17]. can use, microsatellite as a markers in genetic diagnosis because it has many characteristics further that they are abundant and distributed in the genome of the human polymorphic and informative, highly mutagenic, suitable for detecting heterozygotes, experimentally reproducible, cost-effective and easy to detect amplified from low quality and low quantity of DNAs and presumably neutral markers. In addition, microsatellites are very useful for building genetic and genetic maps of many genes in the absence of a reference genome [19]. They are also able to use a few microsatellites markers frequents and a small budget to obtain as many important genetic information as possible. Microsatellites can be also help to cut the time and cost of the study by use many assay such as used for testing non-neutrality and subjected to automated florescent dye-based band scoring through multiplexed genotyping [18]. To detecting the genetics information of the genome by just low marker density especially if LD block sizes of a genome are long with lower costs [20]. While alterations at these sites may be benign if they occur in non-coding regions, they create a recognizable phenotype known as microsatellite instability (MSI) indicating a defect in mismatch repair (MMR) function. Microsatellites markers are excellent indicators of MMR since they are easily genotyped using PCR-based methods, due to their small size [19]. When DNA replication is complete, the template strand will correct itself by incorporating these short tandem repeated nucleotide sequences to match the newly synthesized DNA strand. This allows for a longer or shorter strand of DNA following replication, depending on an insertion or deletion occurred during replication [27].

The advantageous properties of microsatellites have led to modern developments such as digital storage and automated detection and scoring systems such as automated DNA sequences and fluorescent-imaging devices [20]. Under the microsatellite marker assay, can create and develop Universal Premiere to determine the genotype between different types of cancer and genetically analyze distantly
related cancer although there are some limitations and can be a great tool to directly tag and map-based cloning of functionally meaningful “candidate genes” through genotype to phenotype correlations in genetic mapping studies[16]. The abovementioned microsatellite markers were chosen based on historical data which identified defective mismatch repair within the two major MMR genes, namely MSH2 and MLH1 [7]. Mercer et al. and Shen et al. studies used a new methods focused on the role of MSI significance in multiple primary lung cancer they have confirmed the role of MSI at both the early and the late stages of multiple primary lung cancer, and focused on the role of MSI. Additionally, a new method based on the allelic variations at polymorphic microsatellite markers was offered that it does not rely on collection of normal tissue, performed with minimal tumor sample, and the clinical criteria will complement for diagnostic discrimination between metastatic diseases and multiple primary cancers.[1]  

1.6. Disadvantages

In addition to various microsatellite benefits, there is some concerns and disadvantage of it; including the usage of molecular markers is still prohibitively expensive for most large scale application [18]. Microsatellite needs for previous genome sequencing information which that is not available for most prokaryotes (cannot suitable across species), frequently a few number of potential microsatellite loci are identified, polymerase slippage when analyzing mono- and di-nucleotide repeats, co-migrating fragments not always are homologous [21]. Microsatellites are abundant and evenly distributed throughout the genome. In PCR the appearance of the bands are shadows or stutter of short SSR arrays giving multiple bands from single locus. The other concerns of microsatellite are abundance of rare or minor alleles. There is impediment of using microsatellite in case issues with assigning of multiple band SSRs alleles in the absence of correct parentage and pedigree information. Also the size of homoplasy, heteroplasy and cytoplasmic introgression (in particular with cpSSR) due to back mutation during replication slippage [22]. Also microsatellite flanking regions (MFRs) occasionally contain length mutation which may produce identical length variants that could settle microsatellite population level studies and phylogenetic inferences as these length variants in the flanking regions can potentially minimize allele length variation in the repeat region [23]. All of these complicate and bias downstream genetic analysis, it raise f-statistic or p-values, falsify the diversity levels, relatedness, divergence, and true evolution or phylogenetic grouping [24]. Phylogenetic studies should be carried with wariness due to homoplasy and high rate of polymorphism in SSRs for distantly related species [25]. There are several approaches to take into consideration of these caveats when SSRs are used that include verification of size homoplasy, heteroplasy and primer site point mutations using additional cloning and re-sequencing including NGS [24]. in lung cancer it is still a subject of controversy due to its restriction to a small subgroup of patients with high-risk histopathologic features so. The stronger candidate in this category seems to be microsatellite instability (MSI). The recently reported suggest that MSI should be evaluated in order to contribute in treatment decision-making regarding chemotherapy administration [28].

2. Conclusion

Microsatellite Instability Historical Perspective The discovery of microsatellite instability and its association with tumor biology was first established in hereditary non polyposis colon cancer (HNPPCC) in 1993. MSI may be causatively associated with the initiation of molecular changes only, which may later lead to neoplasia which indicates a increasing the accumulation of molecular disorders in cells and a coincidence of mutations in lung cancer. Microsatellites are very powerful genetic markers for identifying cancer and to real diagnostic of lung cancer and pedigree analysis and to study the genetic variation of closely related species and support an expanded role for clinical MSI testing across multiple cancer types. The utilization of microsatellites has been demonstrated by a large number of studies applying this marker and by the variety of areas that apply microsatellites for several purposes. We believe the prognostic value of MSI to be clearer than its predictive value. According to our opinion, MSI status should be evaluated in lung cancer patients in order to contribute in treatment decision-making regarding chemotherapy administration. Microsatellite stable tumors are those that demonstrate no instability at any microsatellite markers tested. One possible way forward is to resolving the problem of prediction and to find easy, appropriate and inexpensive diagnosis of such vital changes to MSI in lung cancer disease is to combine efforts. and analyzed all the large databases which have been found in world especially in USA and Europe preferably to be diagnosed before treatment and after treatment
could allow a per stage stratification and open the field for strong and unambiguous answers regarding prognosis and prediction. Nevertheless, there are some disadvantages that need to be overcome as they hamper the best and widespread use of MSI. The ease of access to the microsatellite, suitability of microsatellite markers for small-scale laboratories with limited budget, the appropriate cost and the strength of the various results made it possible to have genetic diagnosis by the regions of the microsatellites is important and required.

3. Reference

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