Review

Lung Cancer-related Genes in the Blood

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Tumor-related genes can be found circulating in the blood of cancer patients. These genes may be derived from circulating cancer cells or from the patient's primary tumor directly by a process referred to as "gene shedding." Selective and sensitive detection of tumor-related genes in the blood of cancer patients has been made possible by the advent of polymerase chain reaction-based technology that can detect mutations, polymorphisms, microsatellite instability, loss of heterozygosity, and promoter hypermethylation. Several reports have documented the clinical potential of using circulating tumor-related genes as a molecular marker for the early detection of lung cancer, and as a prognostic tool in these patients; larger, prospective studies will be needed to test the feasibility of this approach. Certainly, such an approach in lung cancer patients would be attractive since it is noninvasive and employs relatively easy and rapid methodologies. (Ann Thorac Cardiovasc Surg 2004; 10: 213–7)

Key words: lung cancer, blood, circulating gene, "shed" gene, PCR

Introduction

It has been reported that both free DNA and RNA are present in the blood of cancer patients, and that their concentration increases in patients whose cancer has metastasized.[1-3] The increased presence of circulating DNA and RNA is not in and of itself specific to cancer patients, since it is also found in patients with inflammation, infection, or autoimmune disease such as systemic lupus erythematosus.[4] Recent advances in biochemical technology have made it possible to selectively detect very small amounts of tumor-related genes in the blood including tumor-specific genes, tumor-specific gene alterations, and tumor-specific gene expression. Many of the increasing number of reports in this area have suggested that the ability to selectively detect tumor-related genes in the blood may be useful in the management of cancer patients.

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In this article, we review which tumor-specific genes have been found in circulating blood and discuss the methods used for detecting those genes. We also discuss the clinical potential of this technique in diagnosing lung cancer, providing information necessary in formulating a follow-up strategy, and predicting the patients' prognosis.

Origin of Genes in the Blood

Microsatellite instability (MI), which was demonstrable in small[5,6] and non-small[6-8] cell lung carcinoma tumors, was also found in the circulating DNA of patients with these neoplasms. Allan et al. reported that the loss of allelic heterozygosity (LOH) in circulating genes in patients with lung cancer corresponded to the LOH seen in their primary tumor.[9] Usadel et al. reported that promoter hypermethylation of the APC1A gene was detectable in 47% of the serum/plasma samples of 89 patients with lung cancer that had promoter hypermethylation of the APC1A gene, but not in the sera of 50 healthy controls.[10] Ramirez et al. similarly demonstrated that the patterns of promoter hypermethylation in the serum free DNA of 51 lung cancer patients correlated highly with the patterns seen in the lung cancer cells themselves.[11] However, tumor-related alterations in circulating genes in cancer patients

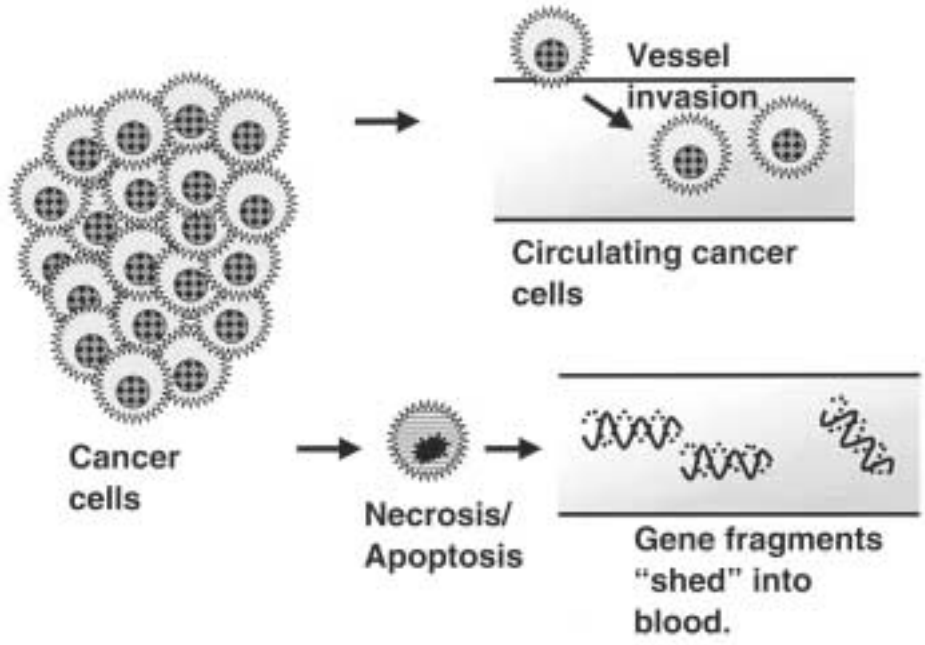

Fig. 1. Two theories as to the source of circulating tumor-related genes.

have not always corresponded to the modification seen in the original tumor.[12,13] Smokers exhibit the same DNA methylation in their normal and precancerous tissues as seen in lung cancer tissue, suggesting that smoking may directly induce the methylation of circulating DNA.[14] Nevertheless, alterations in circulating genes appear to reflect the presence of cancer, having likely originated, at least in the case of lung cancer, from the original tumor.

The presence of cancer-related genes in the blood can most readily be explained in one of two ways[15] (Fig. 1). It may be that these genes originated in circulating cancer cells. Though there is no direct evidence supporting this view in patients with lung cancer, the mRNAs of several genes (e.g., the epidermal growth factor receptor and carcinoembryonic antigen genes), which are rarely detected in healthy persons, have been detected in the blood of lung cancer patients using the reverse transcription polymerase chain reaction (PCR) method.[16-19] These data suggest that circulating lung cancer cells were the source of these mRNAs. Free DNA may also be derived from circulating cancer cells.

Alternatively, tumors may shed their genes into the blood. Some of the cells in primary lung tumors undergo apoptotic or necrotic cell death. As a result, genomic DNA from these cells may enter the circulation, though there is no direct evidence of such "gene shedding." However, Silva et al. did report that the plasma of 44% of breast cancer patients that was collected before mastectomy had some tumor-related DNA alterations, while only 19.5% of patients showed these alterations in plasma that was collected after mastectomy.[20] This reduced rate of DNA alterations after mastectomy indirectly supports the "gene shedding" hypothesis.

Detection of Circulating Tumor-related Genes in the Blood

Several PCR assays, described below, can be used to detect tumor-related genes in the blood.

Detection of mutated genes

Several methods have been used to detect cancer-related gene mutations such as in K-ras codons 12 and 13 and p53. Gene mutations such as point mutations, deletions, and insertions can be detected by single-strand conformation polymorphism analysis,[21] the mutant-enriched PCR method which applies the principle of restriction fragment length polymorphisms,[22] or mutation allele specific PCR.[23]

Detection of MI and LOH

Microsatellites are stretches of variable length DNA that consist of mono-, di-, tri-, tetra-, penta-, or hexanucleotide repeats. While rarely mutated in normal cells,

microsatellites are often mutated in tumor cells where such mutations are referred to as MI. Such instability is a common genetic alteration in lung cancer.[24-26] LOH refers to the deletion of one of two copies of chromosomal allelic DNA sequences. In lung cancer, LOH in regions of chromosomes 3, 5, 9, 13, or 17 is frequently observed.[27-36]

Electrophoresis of the PCR products of microsatellite markers revealed MI in one or more new bands, as well as LOH as revealed by the significant reduction in the density of the allelic band. Chen et al. were the first to report the presence of MI[5] and Sanchez-Cespedes et al. were the first to report LOH analysis in the circulating DNA of lung cancer patients.[7]

Methylation-specific PCR (MSP) for detecting gene promoter hypermethylation

Many human cancers, including lung cancer, exhibit promoter hypermethylation in several tumor suppressor genes. CpG islands i.e., the clustering of CpG dinucleotides in small regions of DNA, are found in promoter regions of almost half of the genes in the genome, including tumor-suppressor genes. Hypermethylation of normally unmethylated cytosines in CpG islands in promoter regions leads to the loss of transcription of the gene which has been referred to as "gene silencing."[37] Promoter hypermethylation of several tumor-suppressor genes including p16ink4a, O6-methylguanine-DNA methyltransferase, death-associated protein kinase, and E-cadherin, among others, is common in lung cancer.[38]

Promoter hypermethylation can be detected using MSP, which was first introduced by Herman et al.[39] In this technique, sample DNA is initially treated with sodium bisulfite to convert unmethylated, but not methylated, cytosines to uracil. PCR with primers specific for methylated DNA can amplify methylated DNA separately from unmethylated DNA, and conversely. This method can detect one methylated copy in 1,000 unmethylated copies, which is a sufficiently high sensitivity for the detection of small amounts of tumor-related genes in blood. Esteller et al.[40] first employed this technique to detect aberrant promoter hypermethylation in tumor suppressor genes in serum DNA from non-small cell lung cancer patients.

Clinical Implications

Detection of tumor-related genes in the blood is a promising new approach that has the advantages of easy access, minimal invasiveness, and rapid analysis,[41,42] though several problems do exist.

Early detection of lung cancer

Whether the detection of tumor-related genes in the blood can be used to diagnose lung cancer at an early stage has not been fully evaluated. By analyzing microsatellite alterations, 40% to 100% of patients with small cell carcinoma were shown to have altered circulating DNA,[5,6,12] while 28% to 77% of patients with non-small cell carcinoma showed such anomalies,[6,7,12,43] Promoter hypermethylation was reported to be present in the circulating DNA in nearly 40% of patients who had lung cancer that displayed promoter hypermethylation.[10,11,44] p53 or K-ras mutations were detected in up to 73% of the circulating DNA in lung cancer patients.[45-47] The frequency of these gene alterations was independent of lung cancer stage suggesting that, even in early stage lung cancer, nearly 40% of patients demonstrate gene alterations in their blood. Thus, detection of alterations in circulating genes may be useful in diagnosing early stage lung cancer, though its low sensitivity rules out its use alone as a screening tool. However, it could be quite useful when used in combination with several markers such as p53 mutations, FHIT LOH, and 3p LOH, as suggested by Andriani et al.[47]

Prediction of prognosis and use as follow-up marker of lung cancer recurrence

Usadel et al. reported a poor prognosis in lung cancer patients who had high levels of methylated APC genes as detected using quantitative MSP.[10] Gonzalez et al. similarly reported a poor prognosis in small cell lung cancer patients who had both a p53 mutation and MI in their plasma DNA.[48] Finally, Ramirez et al. reported a poor prognosis in cancer patients who had K-ras mutations in their serum DNA but no correlation between the patients' methylated serum DNA and their prognosis.[11,49]

While it seems reasonable to speculate that the prognosis of patients who display tumor-related genes in their blood that originated from the primary tumor would be poor, no evidence currently exists to support this view. The significance of "shed" genes on tumor development also remains unclear. Certainly, these are areas that require further investigation.

Gene analysis of blood collected after curative therapy for lung cancer should contribute to the evaluation of this tool as a predictor of prognosis. Usadel et al. reported that lung cancer recurrence could be detected by increased levels of plasma APC methylation though their data were

preliminary.[10] Sozzi et al. showed that high concentrations of DNA in plasma were predictive of lung cancer recurrence.[43] Finally, Silva et al. reported that the persistence of tumor-related DNA in plasma after complete resection of the original breast tumor correlated with histological parameters that were associated with poor outcome.[20] These data suggest that quantifying the concentration of tumor-related genes in the blood may be useful as a follow-up tool for the care of lung cancer patients. Large, prospective studies will need to be undertaken to determine the clinical value of blood gene analysis after curative therapy.

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