Mini Review　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　**Open Access**

BRF2, a Biomarker in Cancer?

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Abstract

Approximately 1.6 million Americans will be diagnosed with cancer in 2014 and 0.6 million Americans will die from cancer, keeping cancer as the second leading cause of death in the United States. These cancer incidence and mortality statistics imply that early detection and intervention are crucial to increasing survival rates. A major obstacle in cancer treatment is the complexity of identification of various combinations of mutations in tumor suppressors and oncogenes in specific cancers and in individuals. It is clear that we have yet to identify all proteins which may have oncogenic potential which can contribute to complications that can arise in designing new individual and tissue specific cancer therapies. Herein, we discuss the role of the RNA polymerase (pol) III specific transcription factor BRF2 in human cancers and its potential use as a biomarker for diagnosis and its potential role in determining appropriate cancer treatment regimens.

Keywords: BRF2; Cancer; Lung cancer; RNA polymerase III; TFIIIB

Introduction

In the United States, it is anticipated that there will be approximately 1.6 million new cancer diagnoses and 0.6 million cancer deaths in 2014, keeping cancer as the second leading cause of death in the United States, second only to heart disease [1]. The death rates for all cancers combined in the United States decreased by 1.8% per year in men and by 1.4% per year in women, as determined from the reported cancer statistics spanning 2006-2010 [1]. These cancer incidence and mortality statistics imply that early detection and intervention are crucial to increasing survival rates. As such, increasing our understanding of the molecular mechanism(s) by which normal cells become deregulated in cancer is a critical component to increasing early intervention strategies.

A major obstacle in the treatment of cancers is the complexity that exists in determining the various combinations of mutations in tumor suppressors and oncogenes in specific cancers and in individuals. It is clear that we have yet to identify all proteins which may have oncogenic potential which can contribute to complications that can arise in designing new individual and tissue specific cancer therapies. As personalized treatment gains popularity with the ability to sequence individual cancers and determine appropriate therapy, it is critical to continue investigating the basic biology which underlies cancer development. Herein, we discuss the role of the RNA polymerase (pol) III specific transcription factor BRF2 in human cancers and its potential use as a biomarker for diagnosis and its potential role in determining appropriate cancer treatment regimens.

RNA pol III transcribes small untranslated RNAs involved in RNA processing (U6 snRNA) and translation (tRNAs) [2]; thereby influencing the growth rate of a cell [3]. Deregulation of RNA pol III transcription can lead to uncontrolled cell growth, a hallmark trait of many types of cancer [4]. For example, cells transformed by chemical carcinogens or DNA tumor viruses have increased RNA pol III products in cancers of the breast, lung, ovary, cervix, and esophagus, but not in matched normal tissues from the same patients (reviewed in [4]). RNA pol III specific transcription factors and products have also been shown to have increased expression in bladder, colon, kidney, or liver carcinomas [5,6].

Accurate initiation from RNA pol III promoters requires TFIIIB [2]. The form of TFIIIB required for proper initiation from gene internal RNA pol III promoters (tRNA) is comprised of TBP, BDP, and BRF1; whereas proper initiation from gene external RNA pol III promoters (U6 snRNA) requires TBP, BDP, and BRF2 [2]. A variety of tumor suppressors have been demonstrated to negatively regulate RNA pol III transcription through TFIIIB as a means of regulating cell growth rates [7], including p53 [8], BRCA1 [9], PTEN [10, 11], RB and the pocket proteins [12]. Oncogenes have also been shown to specifically target TFIIIB such as MYC [13] and the MAP kinase ERK [14]. Additionally, cellular proteins such as Maf1 [15-17] and chemopreventive agents such as EGCG [18] regulate TFIIIB activity in cells.

Amplification of 8p11-1, where the TFIIIB subunit BRF2 is localized, is a well-known alteration in human sporadic breast cancers [19]. Garcia et al. [19] determined there was a correlation between amplification and overexpression after analysis of 33 primary breast tumors, 20 primary ovarian tumors and 27 breast cancer cell lines. This data led to the hypothesis that BRF, positioned at the 8p12 loci, is a candidate oncogene. Interestingly, although 8p11-12 amplification was observed in familial breast cancer, Melchal et al. found that amplification and overexpression of BRF2 did not correlate in familial breast cancer as it did in sporadic breast malignancies in their study [20]. Amplification of 8p11-12 has also been observed in samples of urothelial carcinomas, the bladder cancer cell line JMSU, and correlates with overexpression of BRF2 [21].

The differential expression of the TFIIIB subunits, BRF1 and BRF, at the mRNA level in a variety of cancer cell lines, including: cervical,

prostate, colorectal and breast [5], further, supporting a role for deregulation of TFIIIB-mediated transcription in tumor development [5]. Additionally, it has been demonstrated that the BRF2 promoter is more active than the TFIIIB BRF1 promoter in breast, prostate and cervical cell lines [5].

The observations demonstrating that BRF2 is amplified in various cancers, overexpressed and differentially expressed in a variety of cancer cell lines led researchers to speculate whether there was a correlation and clinical significance regarding BRF2 overexpression in human cancers. Lockwood et al. were the first to characterize BRF2 as an oncogene in squamous cell carcinomas of the lung, a subset of non-small cell lung cancer (NSCLC). Using integrative genomic analysis, they demonstrated that the amplification of chromosome region 8p12 plays a role in the development of 40% of lung squamous cell carcinomas [22]. Furthermore, the authors showed that the oncogene BRF2 was frequently overexpressed in early stages of lung squamous cell carcinoma, including carcinoma in situ and dysplasia. Characterization of BRF2 as an oncogene in lung cancer is of great significance as lung cancer is still the leading cause of death worldwide [1] and NSCLC accounts for 85% of all lung cancers [23].

These observations prompted further investigation by Cabarcas and Schramm to determine if BRF2 was overexpressed in a variety of human cancers in addition to lung cancer using the Oncomine database [6]. The Oncomine database [24] is a cancer microarray database spanning 35 cancer types that contains datasets derived from patient samples, allows investigators to search the microarray data for a particular gene of interest based on any combination of the following criteria: cancer type, cancer versus normal, cancer versus cancer, cancer subtype, cancer versus baseline, pathway and drug and outlier analyses [25]. A query was performed using the Oncomine database to investigate the expression of BRF2 in its datasets and it was determined that BRF2 was specifically overexpressed in human gastric, kidney and melanoma cancers [6]. To determine if BRF2 overexpression correlated with patient outcome in the clinic, researchers focused on breast carcinoma studies analyzing patient death, recurrence and metastasis and noted that BRF2 was found to be overexpressed across 10 breast carcinoma analyses which focused on patient recurrence at 3 and 5 years; metastasis at 1 and 5 years; and patient death at 3 and 5 years [6]. The authors concluded that there is a correlation with BRF2 overexpression and death, recurrence and metastasis in patients diagnosed with breast carcinoma [6]. These data suggested that BRF2 may be a general oncogene in cancer.

In 2014, Toschi et al. evaluated the prognostic relevance of BRF2 copy number alterations in 435 surgically resected stage I and II tissues from NSCLC patients and reported a frequent gain of copy for BRF2. Additionally, the prevalence of a smoking history and squamous cell carcinoma was also significantly associated with BRF2 status [26]. To further investigate the role of BRF2 as an oncogene and as a potential biomarker in NSCLC, Lu et al. analyzed the relationship of BRF2 expression with variable clinicopathologic features and patient prognosis in NSCLC [27]. Samples from 107 patients diagnosed with NSCLC and treated with pulmonary lobectomy plus regional lymph node dissection were analyzed for BRF2 expression in lung tumor samples and compared to adjacent normal lung tissue. This study found an overexpression of BRF2 in these tissues and in addition, found a correlation with BRF2 expression and tumor relapse which was associated with unfavorable overall survival [27].

Recently, Tian et al. explored the possibility of a relationship between the expression of BRF2 protein and the epithelial-mesenchymal transition (EMT) marker proteins in clinical NSCLC samples [28]. EMT, a key step in metastasis, is characterized by epithelial cells losing or acquiring molecular features that change their phenotype from an epithelial cell to a mesenchymal cell phenotype. These key molecular changes involve the inhibition of E-cadherin and an increase in N-cadherin expression, a hallmark trait of EMT [29]. The knockdown of BRF2 expression in NSCLC cells by siRNA led to an increase of E-cadherin and decrease of N-cadherin expression thereby, suppressing the key markers involved in a cell's metastatic ability. Further, the disruption of BRF2 expression significantly inhibited the migratory and invasive abilities of NSCLC cells, suggesting a role for BRF2 in the process of EMT [28]. Univariate analysis demonstrated that BRF2 and E-cadherin protein overexpression accurately predicted a higher risk for recurrence and a decrease in 5-year survival. The authors further analyzed the prognostic value of BRF2 protein expression in a subgroup of patients classified based on the clinicopathological features of NSCLC (T, N, and clinical stage classifications). Using these subgroup of patients, they noted that 5-year survival was significantly decreased in patients expressing high levels of BRF2 expression. Taken together, they concluded that BRF2 protein expression may be an independent NSCLC survival predictor. Thus, it has been speculated that the overexpression of BRF2 in NSCLC might be a prognostic factor of more aggressive NSCLC tumors and a poor clinical outcome [28]. Further, overexpression of BRF2 protein has been detected in 49 out of 91 esophageal squamous cell cancer (ESCC) samples from patients when compared to adjacent and normal tissues [30]. BRF2 protein expression has been shown to be associated with a deeper tumor invasion of esophageal adenocarcinoma. Significantly more microvessel density (MVD) has been detected in tumors with BRF2 protein overexpression, suggesting a possible role of BRF2 protein in tumor angiogenesis. Furthermore, the overall and progression free survival rate of patients with high BRF2 protein expression was significantly lower [30].

The BRF2 expression data in human cancer cells, coupled with TFIIIB activity being regulated by oncogenes and tumor suppressors has led researchers to examine the plausibility of BRF2 overexpression being a common event in human cancers and making BRF2 a potential biomarker in cancer. The recent flurry of clinically relevant BRF2 overexpression data in NSCLC suggests that deregulation of BRF2 may be an early event in the early stages of NSCLC as well as a useful prognostic factor in NSCLC. It is still unclear if BRF2 overexpression is a general feature of all human cancers. However, increasing clinical evidence suggests a role for BRF2 overexpression in breast, gastric, kidney, melanoma and esophageal cancers. Interestingly, to the best of our knowledge, there have not been any reports demonstrating BRF2 mutations in human cancers. We speculate that BRF2 gain of function mutations, single nucleotide polymorphisms, alternatively spliced forms of BRF2 have yet to be reported in human cancers. These genetic alterations have the potential to increase expression of BRF2 in a variety of human cancers. It has been speculated that the c-terminus of BRF2 is responsible for BRF2 mRNA stability [5], and we further argue that point mutations in this region could lead to increased mRNA stability and the clinically relevant BRF2 overexpression observed in a variety of cancers. Sequencing of the BRF2 gene from human cancers, as compared to normal cells, may reveal cancer type specific genetic alterations in BRF2 leading to BRF2 overexpression helping to specify the potential role of BRF2 as a biomarker. Taken together, the data reviewed in this manuscript are extremely significant and demonstrate the potential use of BRF2 as a biomarker

for patients at risk for metastasis. Additionally, the data suggests that BRF2 has the potential to be a therapeutic target. However, the data presented indicate that additional studies are necessary to determine the underlying cause(s) for the observed overexpression of BRF2 before BRF2 can be effectively used as a biomarker in cancer.

Authors' Contributions

LS, SCP and JK reviewed the literature, wrote and finalized the manuscript. All authors read and approved the final version.

Acknowledgements

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