RNA polymerase III transcription in cancer: the BRF2 connection

Stephanie Cabarcas¹ and Laura Schramm²*

Abstract

RNA polymerase (pol) III transcription is responsible for the transcription of small, untranslated RNAs involved in fundamental metabolic processes such as mRNA processing (U6 snRNA) and translation (tRNAs). RNA pol III transcription contributes to the regulation of the biosynthetic capacity of a cell and a direct link exists between cancer cell proliferation and deregulation of RNA pol III transcription. Accurate transcription by RNA pol III requires TFIIIB, a known target of regulation by oncogenes and tumor suppressors. There have been significant advances in our understanding of how TFIIIB-mediated transcription is deregulated in a variety of cancers. Recently, BRF2, a component of TFIIIB required for gene external RNA pol III transcription, was identified as an oncogene in squamous cell carcinomas of the lung through integrative genomic analysis. In this review, we focus on recent advances demonstrating how BRF2-TFIIIB mediated transcription is regulated by tumor suppressors and oncogenes. Additionally, we present novel data further confirming the role of BRF2 as an oncogene, extracted from the Oncomine database, a cancer microarray database containing datasets derived from patient samples, providing evidence that BRF2 has the potential to be used as a biomarker for patients at risk for metastasis. This data further supports the idea that BRF2 may serve as a potential therapeutic target in a variety of cancers.

Introduction

Cancer is a major health problem afflicting millions of Americans annually and despite tremendous research and treatment advances, is still the leading cause of death amongst men and women younger than age 85 years [1]. A dominant characteristic of many types of cancer cells is its ability to proliferate uncontrollably. RNA polymerase (pol) III contains the largest number of subunits (17 subunits) and is responsible for the transcription of small, less than 300 nucleotides, untranslated RNAs involved in fundamental metabolic processes, such as RNA processing (U6 snRNA) and translation (tRNAs), which contribute to cell proliferation [2]. Thus, deregulation of RNA pol III transcription can lead to aberrant production of critical RNAs contributing to uncontrolled cell growth, a hallmark trait of many types of cancer.

Like all eukaryotic polymerases, RNA pol III cannot recognize its target promoters directly and accurate initiation requires TFIIIB [2-4]. In higher eukaryotes, thus far, two forms of TFIIIB have been identified [2-4]. BRF1-TFIIIB required for transcription by gene internal RNA pol III promoters (tRNA) contains Bdp1, TBP and BRF1 (Figure 1). BRF2-TFIIIB required for transcription from RNA pol III gene external promoters contain Bdp1, TBP and BRF2 (Figure 1) [2]. Examples of genes transcribed by BRF2-TFIIIB include the human U6 snRNA gene involved in RNA splicing, the 7SK gene whose RNA product has been demonstrated to negatively regulate RNA Pol II transcription elongation by binding to the elongation factor P-TEFb, the RNase mitochondrial RNA processing (MRP) which participates in pre-rRNA processing, novel noncoding RNAs of unknown function (reviewed in [2,5]).

BRF2 (TFIIB-related factor 2) shares structural features with TFIIIB and BRF1 (Figure 1B). TFIIIB, BRF1 and BRF2 all contain N-terminal zinc ribbon domains, core domains containing imperfect repeats; BRF1 and BRF2 have unrelated C-terminal extensions (Figure 1B) [2]. The C-terminus of BRF2 is required for association with TBP and SNAPc (small nuclear activating protein complex) on the U6 promoter [6].

* Correspondence: schramml@stjohns.edu
²Department of Biological Sciences, St. John’s University, Queens, New York 11439, USA
Full list of author information is available at the end of the article

© 2011 Cabarcas and Schramm; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Many different transformed cell types have been shown to have increased products of RNA pol III, when transformed by DNA tumor viruses, as well as chemical carcinogens [7-11] and their relevance has been validated in tumors of the breast, cervix, esophagus, lung, ovary, parotid, and tongue, but not in corresponding normal tissues tumors [12]. Specifically, RT-PCR analysis has demonstrated that tRNAs are overproduced consistently in human ovarian cancers [13]. Also, tRNA levels have been shown to be 10-fold higher in breast cancer cells than in normal cells [14]. These increases are not simply a consequence of rapid cell proliferation in cancer [15], but instead contribute to tumorigenesis, as it has been demonstrated that overexpression of tRNAiMet induces proliferation and immortalization of fibroblasts [16].

Amplification of BRF2 has been noted in breast cancer [18,19] and more recently a human bladder cancer cell line [20]. Recently, Lockwood et al. demonstrated that genetic activation of BRF2 represents a unique mechanism of squamous cell carcinoma tumorigenesis, also providing the first clinical evidence implicating BRF2 as a novel lineage-specific oncogene in lung squamous cell carcinoma [20]. This review will focus on BRF2-TFIIIB activity in cancer.

**Regulation of BRF2-TFIIIB activity by oncogenes and tumor suppressors**

RNA pol III transcription is tightly regulated during the cell cycle to ensure normal cellular growth [21]. Cellular levels of RNA pol III are specifically increased in tissues isolated from mice with myeloma compared to tumor-free mice [22], directly linking RNA pol III activity and cancer. Recently, it was demonstrated that BRF1 and TBP are capable of driving oncogenic transformation [16,23]. These observations demonstrate that elevation of RNA pol III transcription contributes to oncogenesis. TFIIIB activity is strictly regulated by Maf1 [24-27], chemopreventative agents [28], and oncogenes and tumor suppressors which are discussed below.
CK2 (casein kinase 2) is a ubiquitous and conserved protein kinase with growth-promoting and oncogenic properties. CK2 is abnormally active in a variety of human cancers (Figure 2) [29]. It has been demonstrated that CK2 interacts stably with TFIIIB; BRF1 is phosphorylated in cells and CK2 inhibitors can decrease this phosphorylation, thereby promoting transcription complex assembly [29].

TFIIIB activity is targeted by tumor suppressors [30-36] such as ARF [37], RB (Retinoblastoma protein) [30,38], p53 [39-43] and BRCA1 (breast cancer susceptibility gene 1) [44].

RB controls cell growth by preventing cell cycle entry in the absence of appropriate mitogenic signals and inactivation is associated with a variety of human cancers [45]. RB regulates RNA pol III transcription by disrupting interactions between TFIIIB and RNA pol III [38,46-49] RB-mediated repression of U6 transcription can be restored by recombinant SNAPc and TBP [46].

p53 is activated in response to cellular stress, inducing cell cycle arrest or apoptosis, and its inactivation is considered a critical step in carcinogenesis [50]. p53 represses not only Alu and U6 transcription, but also tRNA, 5S rRNA, VAI, B2 and EBER (Epstein-Barr virus) transcription, establishing p53 as a general repressor of RNA pol III transcription [39]. p53 regulates U6 transcription through interaction with the BRF2-TFIIIB subunit TBP [41] and SNAPc [51].

BRCA1 plays a role in DNA repair, cell cycle regulation, apoptosis, genome integrity and ubiquitination [52,53]. Recently, BRCA1 has been characterized as a general repressor of RNA pol III transcription [44]. BRF2 overexpression alleviates BRCA1 mediated repression of U6 transcription [44], suggesting that regulation of U6 transcription by BRCA1 occurs, in part, through BRF2. However, it is currently unclear whether the observed inhibition of RNA pol III transcription is a result of direct or indirect interactions between BRCA1 and BRF2, or BRCA1 and TFIIIB in general.

**BRF2, a general oncogene?**

It is established that RNA pol III is often deregulated in cancers [33-35] and specific elevation of RNA pol III transcripts and RNA pol III transcription factors such as U6 snRNA and BRF2 is a feature of both transformed cells and cancers [54]. Recently, Lockwood et al identified BRF2 as a novel oncogene in lung squamous cell carcinoma demonstrating that overexpression of BRF2 can drive expression of RNA pol III transcripts contributing to squamous cell carcinoma tumorigenesis [20]. However, it cannot currently be ruled out that TFIIIB, particularly the BRF2 subunit, could bind and potentially titrate tumor suppressors, thus alleviating some key mechanisms normally keeping TFIIIB activity in check, contributing to oncogenesis. Additionally, no Brf2-dependent pol III transcript has yet been shown to have transforming activity.

RNA pol III is a fundamental determinant of the capacity of a cell to grow and the identification of BRF2 as an oncogene further demonstrates the importance of proper regulation of RNA pol III transcription. Hence, we queried the Oncomine database to systematically assess gene expression levels of BRF2 in a variety of carcinomas. Oncomine is a bioinformatics initiative which collects, standardizes, analyzes, and delivers cancer transcriptome data to the biomedical research community [55]. Rhodes et al. analysis of cancer transcriptome data has identified the genes, pathways, and networks deregulated across 18,000 cancer gene expression microarrays spanning 35 cancer types (for a comprehensive overview of the Oncomine database refer to [55]). Differential expression analysis is an important feature of the Oncomine resource. A unique feature of the Oncomine database is Oncomine automatically computes differential
expression profiles for cancer types and subtypes allowing for simple query for individual gene expression.

Thus, using the Oncomine database, we performed a disease summary for BRF2 (Figure 3) to determine if BRF2 overexpression was significant in various carcinomas. Based on this analysis, we determined that in studies comparing cancer versus normal tissue, BRF2 is highly overexpressed in datasets focused on gastric, kidney and melanoma cancers. Please note that sample number for each data set used in our BRF2 analysis is noted, and data sets are named for author whose data set has been analyzed. In Figure 3, overexpression represented by ‘red cells’ and under expression represented by ‘blue cells’ is determined based on the gene rank percentile. The outlier analysis, as demonstrated in Figure 3, suggests that there are 50 analyses which have a significant increase in BRF2 expression and 45 analyses which have a significant decrease in BRF2 expression. The outlier analysis represents a small sub-population of samples within the datasets, hence, they do not reflect the majority of samples. We speculate that out of the 478 unique analyses, the 45 analyses which have a significant decrease of BRF2 expression demonstrates that BRF2 overexpression may not be universal to all cancer patients. The Oncomine database is a compilation of gene expression studies performed from clinically-based analyses performed on patient samples. The 4 analyses which are significant out of 162 that are significant include gastric, kidney and melanoma cancer datasets (Figure 3). This criterion for this specific BRF2 disease summary performed was stringent as we required a p-value of 1E-4 and a fold-change of 2 for BRF2 gene expression compared to the controls. Hence, we believe that due to the stringency of our criterion, only 4 analyses showed a significant increase in BRF2 expression.

**Figure 3 Oncomine Analysis: Disease Summary for BRF2** The Oncomine database was queried for BRF2 expression in the available datasets based on the following: cancer type, cancer versus normal, cancer versus cancer, cancer subtype, cancer versus baseline, pathway and drug and outlier analyses. The ‘red cells’ represents BRF2 overexpression and the ‘blue cells’ represent BRF2 underexpression. The levels of expression are based on the gene rank percentile. This disease summary was performed using a criterion of a 2 fold change for BRF2 expression and a p-value of 1E-4. Using these stringent criteria for BRF2 expression analysis, we found that BRF2 is highly overexpressed in the following cancer vs normal datasets: gastric, kidney and melanoma cancers. In the cancer vs cancer, multi-cancer datasets, BRF2 is overexpressed in leukemias and lymphomas. The outlier analysis, which is used to determine significant BRF2 expression in a subset of the patient samples, showed that BRF2 is both over- and under-expressed across the analyzed cancers. (Oncomine database).
We further queried the analyses which had a significant increase in BRF2 expression and as demonstrated in Figure 4A, the Talantov Melanoma dataset, comprised of 70 patient samples shows a statistically significant overexpression of BRF2 mRNA, p-value = 1.15E-15, in cutaneous melanoma compared to normal skin. Figure 4B shows a statistically significant overexpression of BRF2 mRNA, p-value = 7.64E-5, in clear renal cell carcinoma compared to normal kidney in the Gumz Renal dataset, comprised of 20 patient samples. Lastly, Figure 4C shows a statistically significant overexpression of BRF2 mRNA, p-value = 5.35E-5, in the DErrico Gastric diffuse gastric adenocarcinoma compared to gastric mucosa dataset, comprised of 69 patient samples. Additionally, Lockwood et al have recently identified BRF2 as an oncogene in lung squamous cell carcinoma [20]. To determine if there was a correlation with BRF2 overexpression and lung carcinoma, we analyzed the Garber Lung dataset, comprised of 73 patient samples, Figure 4D. The data retrieved from the Garber Lung study shows a correlation between BRF2 overexpression and an advanced N stage, N1, in lung carcinoma samples.

Figure 4 Oncomine analysis of BRF2 expression in gastric, kidney, melanoma and lung cancers. As seen in Figure 3, BRF2 is significantly overexpressed in gastric, kidney and melanoma cancer datasets within the Oncomine datasets. We further queried Oncomine for each of these specific cancers to compare the fold change of BRF2 expression in the cancerous tissue compared to the controls for each specific cancer. All p-values represent a student’s t-test. Figure 4A shows that BRF2 is overexpressed in cutaneous melanomas compared to normal skin in the Talantov Melanoma dataset [58]. BRF2 overexpression is significant with a p-value of 1.15E-15 and a total of 70 patient samples used for this analysis. Figure 4B shows that BRF2 is overexpressed in clear cell renal carcinoma compared to normal kidney in the Gumz Renal dataset [59]. BRF2 overexpression is significant with a p-value of 7.64E-5 and a total of 20 patient samples were used for this analysis. Figure 4C shows that BRF2 is overexpressed in diffuse gastric adenocarcinoma versus normal gastric mucosa in the DErrico Gastric dataset [60]. BRF2 overexpression is significant with a p-value of 5.35E-5 and a total of 69 patient samples were used for this analysis. Lastly, Figure 4D shows that BRF2 is overexpressed in advanced N1+ stage lung adenocarcinoma compared to N0 stage in the Garber Lung Adenocarcinoma dataset [61]. BRF2 overexpression is significant with a p-value of 0.023 and a total of 73 patient samples were analyzed. (Oncomine database).
All statistical values relative to these analyses were calculated as previously described [55].

Further query of the information presented in the BRF2 disease summary demonstrated that in analyses comparing multi-cancers, “cancer versus cancer”, BRF2 is overexpressed in both leukemia and lymphoma as well. In the case of BRF2 expression in leukemia, we searched various leukemia datasets and found that across 22 analyses, BRF2 was significantly overexpressed, Figure 5A, p-value = 0.028. In the case of BRF2 expression in lymphoma, we found that across 4 analyses, BRF2 was significantly overexpressed, Figure 5B, p-value = 0.011.

Interestingly, analysis of the BRF2 disease summary shows that BRF2 is highly expressed on the basis of outlier gene expression using a method called COPA (cancer outlier profile analysis). COPA was previously described and utilized to identify oncogenic chromosomal aberrations such as the TMPRSS2:ETS fusion gene in prostate cancer by Tomlins et al [56]. Based on the BRF2 disease summary, we focused on breast carcinoma as this was most significant. Analysis of the 95% outlier across 17 breast carcinoma analyses (Figure 6) shows that BRF2 overexpression is highly significant in a small sub-population of samples.

Lastly, we investigated if there was a correlation between BRF2 overexpression and clinical outcome. Using breast carcinoma studies, we performed a meta-analysis across 10 different breast carcinoma datasets studying recurrence, metastasis and death, Figure 7A. We determined that BRF2 overexpression did indeed correlate with clinical outcome. Figure 7B represents one dataset analyzed in Figure 7A, the vandeVijver breast carcinoma study, and shows that BRF2 overexpression is highly significant in patients presenting with metastasis at year 1, p-value = 0.034, and at year 5,
p-value = 0.006. This data set is extremely significant at it shows promise for the potential use of BRF2 as a potential biomarker for patients at risk for metastasis and may serve as a potential therapeutic target.

Recently, RNA pol III transcription has been the focus of a Phase I and pharmacokinetic study. Hammond-Thelin et al. studied the effects of a novel nucleoside analog inhibitor (TAS-106) of RNA pol I, II and III, in patients with advanced solid malignancies [57]. Previously, TAS-106 has demonstrated antitumor activity in various human cancer models including leukemic, lung, colorectal, stomach, pancreatic, and gastric cancers [57]. The principal objectives of the study were to determine the maximum tolerated dose in patients, characterize the toxicities associated with TAS-106 administration, determine the pharmacokinetics of TAS-106 and study if there was any indication of antitumor activity in patients [57]. Although this study is in its infancy, it’s representative of the potential use of RNA pol III inhibitors as a means of pharmacological target for the treatment of cancers.

Conclusions
By elucidating the mechanism(s) by which RNA pol III transcription is both regulated and deregulated, it will be possible to further understand the mechanism(s) by which aberrant activity of the general transcription machinery contributes to cancer development. Deregulation of RNA pol III transcription in cancers coupled with the observation that TFIIIB, specifically BRF2-TFIIIB, is commonly a target of deregulation in a variety of cancers demonstrates that RNA pol III transcription is indeed a key player in tumorigenesis and could serve as a novel target in the development of pharmacological agents.
Acknowledgements

This work was supported in part by NIH grant 1R15CA133842-01A1 (LS). The authors wish to apologize, due to space restrictions, that not all TFIIIB studies could be mentioned. We thank Dr. Joby Jacob for his assistance with figure preparation.

Author details

1 National Cancer Institute, Laboratory of Cancer Prevention, Cancer Stem Cell Section, 1050 Boyles Street, Building 560, Room 21-81, Frederick, MD 21702, USA. 2 Department of Biological Sciences, St. John’s University, Queens, New York 11439, USA.

Authors’ contributions

SC reviewed the literature, wrote and drafted the manuscript; LS corrected and finalized the manuscript. All authors read and approved the final version.

Competing interests

The authors declare that they have no competing interests.

Received: 13 December 2010 Accepted: 25 April 2011 Published: 25 April 2011

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009, 59(4):225-49.
2. Schramm L, Hernandez N. Recruitment of RNA polymerase III to its target promoters. Genes Dev 2002, 16(20):2593-620.
3. Huang Y, Maraia RJ. Comparison of the RNA polymerase III transcription machinery in Schizosaccharomyces pombe, Saccharomyces cerevisiae and human. Nucleic Acids Res 2001, 29(13):2675-90.
4. Geiduschek EP, Kassavetis GA. The RNA polymerase III transcription apparatus. J Mol Biol 2001, 310(1):1-26.
5. Dieci G, Finorino G, Castelnuovo M, Techmann M, Pagano A: The expanding RNA polymerase III transcriptome. Trends Genet 2007, 23(12):614-22.

6. Saxena A, Ma B, Schramm L, Hernandez N: Structure-function analysis of the human TFIIIB-related factor II protein reveals an essential role for the C-terminal domain in RNA polymerase III transcription. Mol Cell Biol 2003, 23(11):9406-18.

7. Wang HD, Yuh CH, Dang CV, Johnson DL: The hepatitis B virus X protein increases the cellular level of TATA-binding protein, which mediates transcriptionactivation of RNA polymerase III genes. Mol Cell Biol 1995, 15(12):7208-15.

8. White RJ, Scott D, Rigby PW: Regulation of RNA polymerase III transcription in response to Simian virus 40 transformation. Embryo J 1990, 9(11):3713-21.

9. Gottfeld MD, Johnson DL, Nyberg J: Transcriptional activation of RNA polymerase III-dependent genes by the human T-cell leukemia virus type I tax protein. Mol Cell Biol 1996, 16(4):1777-85.

10. Larminie CG, Cairns CA, Mital R, Martin K, Kouzarides T, Jackson SP, Larminie CG, Cairns CA, Sutcliffe JE, Winter AG, Dieci G, Fiorino G, Castelnuovo M, Teichmann M, Pagano A, Schramm L: Structure-function analysis of enhanced RNA polymerase III transcription by RB, p53 and c-Myc. Mol Cell Biol 2002, 22(11):3575-88.

11. Larminie CG, Cairns CA, Sutcliffe JE, Winter AG, Winter AG, Dieci G, Fiorino G, Castelnuovo M, Teichmann M, Pagano A, Schramm L: Structure-function analysis of enhanced RNA polymerase III transcription by RB, p53 and c-Myc. Cell Cycle 2003, 2(3):181-4.

12. Marshall L, White RJ: Non-coding RNA production by RNA polymerase III is implicated in cancer. Nat Rev Cancer 2008.

13. Mueller EA, Scott D, Cabrera J, Cabrera J, Morris GR, McGowan DS, Williams JG, Sutrathod P, Dang CV, Rine J: Transcription by Maf1 in Mammalian Cells. J Biol Chem 1997, 272(26):16254-60.

14. Pavon-Eternod M, Gomes S, Geslain R, Dai Q, Rosner MR, Pan T: Identifying new candidate oncogenes. Oncogene 2008, 27:2506-16.

15. Rollins J, Veras I, Cabreras S, Willis I, Schramm L, Grandon C, Eisenman RN, White RJ: Direct regulation of RNA polymerase III transcription by RB, p53 and c-Myc. Cell Cycle 2003, 2(3):181-4.
55. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, Barrette TR, Anstet MJ, Kincaid-Beal C, Kulkarni P, Varambally S, Ghosh D, Chinnaiyan AM: Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia* 2007, 9(2):166-80.

56. Tomlinson GE, Chen TT, Stastry VA, Virmani AK, Spillman MA, Tonk V, Blum JL, Schneider NR, Wistuba II, Shay JW, Minna JD, Gazdar AF: Characterization of a breast cancer cell line derived from a germ-line BRCA1 mutation carrier. *Cancer Res* 1998, 58(15):3237-42.

57. Hammond-Thelin LA, Thomas MB, Iwasaaki M, Abbruzzese JL, Lassere Y, Meyers CA, Hoff P, de Bono J, Norris J, Matisuhita H, Mita A, Rowinsky EK: Phase I and pharmacokinetic study of 3'-C-ethynylcytidine (TAS-106), an inhibitor of RNA polymerase I, II and III, in patients with advanced solid malignancies. *Invest New Drugs* 2010.

58. Talantov D, Mazumder A, Yu JX, Briggs T, Jiang Y, Backus J, Atkins D, Wang Y: Novel genes associated with malignant melanoma but not benign melanocytic lesions. *Clin Cancer Res* 2005, 11(20):7234-42.

59. Gumz ML, Zou H, Kreinest PA, Childs AC, Belmonte LS, LeGrand SN, Wu KJ, Luxon BA, Sinha M, Parker AS, Sun LZ, Ahquista DA, Wood CG, Copland JA: Secreted frizzled-related protein 1 loss contributes to tumor phenotype of clear cell renal cell carcinoma. *Clin Cancer Res* 2007, 13(16):4740-9.

60. D’Errico M, de Rinaldis E, Blasi MF, Viti V, Falchetti M, Calcagnile A, Sera F, Saieva C, Ottini L, Palli D, Palombo F, Giuliani A, Dogliotti E: Genome-wide expression profile of sporadic gastric cancers with microsatellite instability. *Eur J Cancer* 2009, 45(3):461-9.

61. Kickhoefer VA, Searles RP, Kedersha NL, Garber ME, Johnson DL, Rome LH: Vault ribonucleoprotein particles from rat and bullfrog contain a related small RNA that is transcribed by RNA polymerase III. *J Biol Chem* 1993, 268(11):7868-73.

doi:10.1186/1476-4598-10-47

Cite this article as: Cabarcas and Schramm: RNA polymerase III transcription in cancer: the BRF2 connection. *Molecular Cancer* 2011 10:47.