MYC Synergizes with Activated BRAFV600E in Mouse Lung Tumor Development by Suppressing Senescence

Vedrana Tabor¹,², Matteo Bocci¹,³, Nyosha Alikhani¹, Raoul Kuiper⁴, and Lars-Gunnar Larsson¹

Abstract

The activated RAS/RAF cascade plays a crucial role in lung cancer, but is also known to induce cellular senescence, a major barrier imposed on tumor cells early in tumorigenesis. MYC is a key factor in suppression of RAS/BRAFV600E-induced senescence in vitro. However, it is still unclear whether MYC has the same role during tumor development in vivo. Using a conditional, compound knock-in model of Cre-activated BRAFV600E and tamoxifen-regulatable MycER, we show that tamoxifen-induced activation of MYC accelerated the onset and increased the number and size of BRAFV600E-driven adenomas in a dose-dependent manner, resulting in reduced survival. Furthermore, MYC activation leads to reduced expression of the senescence markers p16INK4A, p21CIP1, and H3K9me3-containing heterochromatin foci, and an increased percentage of Ki67+ tumor cells. This suggests that MYC already early during tumor formation suppresses a BRAFV600E-induced senescence-like state. Initial activation of MYC followed by tamoxifen withdrawal still resulted in an increased number of tumors and reduced survival. However, these tumors were of smaller size, showed increased expression of p16INK4A and p21CIP1, and reduced number of Ki67+ cells, indicating that MYC inactivation restores BRAFV600E-induced senescence. Surprisingly, MYC activation did not promote adenoma to carcinoma progression. This suggests that senescence suppression by MYC is a discrete step in tumor development important for sustained tumor growth but preceding malignant transformation and that additional oncogenic events are required for carcinoma development and metastasis. These findings contribute to our understanding of the neoplastic transformation process, with implications for future treatment strategies. Cancer Res; 74(16): 4222–9. ©2014 AACR.

Introduction

Lung cancer is a leading cause of cancer-related death worldwide. Recent genome-wide analysis of driver mutations in non–small cell lung cancers have identified BRAF mutations in 9% of the pulmonary adenocarcinoma subset, with V600E (2%-3%) as the most common (1), correlating with poor prognosis (2). BRAF encodes for a Ser/Thr kinase playing a central role as a RAS effector in the RAS–RAF–MAPK signaling pathway, which connects extracellular signals to transcriptional regulation of genes involved in proliferation and cancer development. At least 25% of all mutations in pulmonary adenocarcinoma target this pathway (1). Another central player in tumorigenesis is the transcription factor MYC, which is rearranged and/or overexpressed in over half of human cancers (3), including lung adenocarcinomas where it is found amplified in 30% of cases (1), correlating with poor prognosis (4).

Mutated, activated RAS or BRAF is known to trigger oncogene-induced senescence (OIS) in preneoplastic lesions in lung adenomas (5, 6). Cellular senescence is a state of irreversible proliferation arrest, which together with apoptosis forms two of the main barriers against tumor development and usually relies on the p53/CDKN1A (p21CIP1) and CDKN2A (p16INK4A)/pRB tumor suppressor pathways (6). We and others have shown that the MYC oncogene suppresses RAS/BRAF-induced senescence in normal primary fibroblasts and in tumor cells in vitro (7, 8), although it can also induce OIS under certain conditions (6, 9). However, whether it plays a role in senescence suppression in vivo has not yet been demonstrated. To address this question, we utilized a mouse model of BRAFV600E-induced lung adenoma characterized by prominent senescence-like growth arrest (5), intercrossed with mice containing a regulatable MycER allele (10). Our results show that MYC synergizes with BRAFV600E in lung tumorigenesis by suppressing the senescence-like state, correlating with increased number and size of tumors with elevated proliferative capacity, resulting in reduced survival of the compound mice.

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Materials and Methods

Mice

All animal protocols in this study were approved by the ethical committee of the Swedish Board of Agriculture. BRAFV600E conditional knock-in mice (BrafC; ref. 5) were intercrossed to conditional, regulatable MycER knock-in mice (R26-lsl-MERT2; ref. 10) and maintained on a mixed C57BL6/FVBN background. Generation of heterozygous and homozygous offspring was verified by genotyping (Supplementary Fig. S1A). Monitoring of adenoma onset was carried out as described previously (5, 10). Animals were euthanized at specific time points or upon display of visible signs of disease.

Adenoviral Cre delivery and tamoxifen regimen

Ad-Cre-GFP and Ad-empty-GFP were obtained from the Gene Transfer Vector Core, University of Iowa (Iowa City, IA). Six- to 8-week-old mice were exposed to 5 × 10^7 plaque-forming unit through nasal inhalation of a calcium phosphate precipitate (5). Of note, 1 mg/20 g body weight of tamoxifen was administered orally once per day, starting 7 days after virus precipitation (5). Of note, 1 mg/20 g body weight of tamoxifen was administered orally once per day, starting 7 days after virus inhalation until sacrifice of the mice, except in MYC on/off experiments where tamoxifen treatment was discontinued after 7 days.

Immunoblotting, IHC, immunofluorescence, and microscopic analysis

PBS-flushed whole lungs were either fixed in 4% paraformaldehyde and paraffin embedded or snap frozen. Whole protein lysate from PBS-flushed lungs was prepared as described previously (14). Tissue sections were stained with hematoxylin and eosin (H&E) to determine tissue and tumor morphology. Tumor grades and proliferative status in lung tissues sections as described in ref. 11. Apoptosis was detected with previously published protocol described in ref. 11.

The following antibodies were used for immunoblot analysis and immunostaining: c-MYC (sc-42), CC-10 (sc-9772), SP-C (sc-11008) and rabbit anti-mouse (A-11059; all from Invitrogen), AlexaFluor 488 goat anti-rat (A-1106), goat anti-rabbit (A-11088) and rabbit anti-mouse (A-11098; all from Invitrogen), and biotinylated rabbit-anti-mouse (E046401-2) from DAKO. Fluorescence signals were detected by a laser-scanning microscope (Axiovert 200 M; Zeiss).

For quantification of nuclear Ki67 signal, tissue sections were scanned with a 3D Histech Panoramic Midi scanner and analyzed with 3D Histech Panoramic Viewer software, using their Nuclear Quant module.

Senescence and apoptosis assays

Lysosomal β-galactosidase activity was assayed on frozen tissue sections as described in ref. 11. Apoptosis was detected on paraffin-embedded sections with the In Situ Cell Death Detection Kit, Fluorescein (Roche), following the manufacturer’s instruction.

Statistical analysis

Data were analyzed with GraphPad Prism software by one-way ANOVA using Bonferroni correction for post hoc tests. Survival between the diverse groups was compared using the log-rank (Mantel-Cox) test.

Results

MYC activation shortens survival during BRAFV600E-induced lung tumor development in a dose-dependent manner

To determine the impact of MYC activity on BRAFV600E-driven tumorigenesis, we intercrossed mice containing Cre-inducible BRAFV600E (5) and MycER (R26-lsl-MERT2; ref. 10) transgenes. In addition, the MycER protein is regulatable by tamoxifen. BRAFV600E and MycER recombination in mice was induced by intranasal Ad-Cre-GFP delivery, whereas no recombination occurred in control animals receiving Ad-empty-GFP. The following groups of mice were generated: BRAFV600E (B^V600E), BRAFV600E, MycER^LKI (BM^LKI), BRAFV600E, MycER^WT/KI (BM^WT/KI), as well as controls Braf^wild-type (B^WT), MycER^WT/KI (A^WT/KI), and MycER^LKI (A^LKI).

Tamoxifen-treated cohorts of compound mice developed tumors significantly faster than those receiving vehicle (peanut oil); BM^LKI and BM^WT/KI mice had a median survival time of 31 and 43 days, respectively, compared with vehicle-treated BM^WT/KI mice (73 days; Fig. 1A). Vehicle-treated BM^WT/KI or tamoxifen-treated B^WT cohorts displayed similar survival to B^VT mice (data not shown). Tamoxifen-treated B^WT animals or B^WT/KI mice did not develop tumors over a period of 100 days (Fig. 1A and data not shown). This suggests that activation of MYC is not tumorigenic per se within 100 days, but significantly shortens survival of BRAF-induced tumors in a dose-dependent manner even with a modest increase in expression of approximately 2-fold (Supplementary Fig. S1B).

MYC activation results in increased number and size of BRAFV600E-induced lung tumors

We observed a 2-fold increase in the lung-to-total body weight ratio in vehicle-treated BM^WT/KI mice compared with control (wild-type) B^WT and vehicle-treated BM^WT/KI lungs (Supplementary Fig. S1C). Tamoxifen-treated BM^LKI and BM^WT/KI showed an additional 1.5-fold increase. Furthermore, tamoxifen-treated BM^WT/KI and in particular BM^LKI showed a significantly increased number and size of tumors compared with vehicle-treated BM^WT/KI (Fig. 1B and C), resulting in a higher ratio between the tumor area and the total lung area, as shown by H&E staining of whole lung sections (Fig. 1B).

When compared with the healthy lungs, surfactant protein-C (SP-C) expression was increased in lungs of tumor-bearing animals, whereas Clara cell antigen expression was reduced (Supplementary Fig. S1D). This suggested an AT2PC (alveolar type 2 progenitor cells) phenotype of the tumor, consistent with previously published findings (5). Histopathological analysis showed that the lungs of vehicle-treated BM^WT/KI mice variably showed mild bronchiolar hyperplasia, and relatively small, circumscribed papillary adenomas. Foci with a more solid growth pattern were occasionally observed (Fig. 1D), but
Figure 1. MYC activation increases number and size of BRAFV600E-induced lung tumors in mice and decreases overall survival. A, Kaplan–Meier survival plot of vehicle-treated (VT) \textit{BrafV600E}; \textit{MycER} WT/KI (BMWT/KI; \( n = 11 \), red), tamoxifen (TAM)-treated BMKI/WT (\( n = 31 \), blue), and \textit{BrafV600E}; \textit{MycER} KI/KI (BMKI/KI; \( n = 11 \), green) mice. In addition, control \textit{BrafCA} mice given empty Ad vector by inhalation and treated with tamoxifen are depicted in black (\( n = 8 \)). Gray box, tamoxifen or vehicle treated (started at day 7 post-inhalation until time of death). B, quantitative analysis of average number of tumors per lung section (left) and total tumor area per total lung area (right) of indicated genotypes and treatments (BMWT/KI VT, \( n = 3 \); BMWT/KI TAM, \( n = 4 \); BMKI/KI TAM, \( n = 3 \); BMWT/KI TAM on/off, \( n = 2 \), two sections per lung). Shown are mean \pm SEM. Statistically significant differences from the control are shown \( * \), \( P < 0.05 \); \( ** \), \( P < 0.01 \). C, pie charts showing the average number of tumors as analyzed in B, distributed over four different arbitrary set tumor sizes (areas, representing number of pixels \( \times 10^3 \) relative to the total lung area) for the indicated genotypes and treatments. D, H&E-stained lung sections showing representative tumor lesions in BM mice of indicated genotypes treated with vehicle or tamoxifen as compared with control mice. Top, middle, and bottom images at lower magnification, \( \times 5 \) and \( \times 10 \), respectively. Bars, 50 \( \mu \)m. The analyses in B–D were done at endpoints when animals were sacrificed because of disease.
hallmarks of advanced adenoma (such as nuclear pleomorphism, prominent nucleoli, mitoses, or necrosis) were rare or absent. Tamoxifen-treated mice exhibited more pronounced bronchiolar hyperplasia and larger adenomas, with accordingly larger areas showing mucoid differentiation and vesicular nuclei, consistent with a bronchiolar component also in these tumors (Fig. 1D).

Although the tumors were larger, particularly in BMKI/KI mice, as well as local indications of tumor progression, there were no consistent indications of increased frequency of adenoma to carcinoma transition in response to MYC activation. Furthermore, we did not observe metastatic spread in spleen, brain, or other organs in any of the genotypes or treatments (data not shown), although the presence of micrometastases cannot be excluded.

Taken together, we conclude that MYC activation resulted not only in shortened survival of mice with activated BRAFV600E, but also in increased number and size of BRAFV600E-induced lung adenomas.

**MYC activation suppresses hallmarks of BRAFV600E-induced senescence in lung tumors**

We next assessed the impact of MYC on BRAFV600E-induced senescence as a potential factor for its acceleration of lung tumor development. Sporadic senescence-associated β-galactosidase (SA-β-gal) activity observed in wild-type lungs was significantly enhanced with the expression of BRAFV600E in tamoxifen and vehicle-treated BMWT/KI and BMWT/KI mice at the time of sacrifice due to disease. However, the staining patterns were often heterogeneous (Supplementary Fig. S2A). To obtain a clearer picture of the distribution of senescent cells within tumors, we investigated the expression of CDKN2A/p16INK4A, another senescence marker. A significant reduction in the relative expression levels of p16INK4A was observed in tumors of tamoxifen-treated BMKWT and BMK/Wt mice compared with vehicle-treated BMWT/KI mice (Fig. 2A and B). Furthermore, foci of trimethylated histone H3 lysine 9 (H3K9me3), yet another marker of senescence, which partially colocalized with p16INK4A, were also reduced in the tumor areas of tamoxifen treated compared with vehicle-treated BM animals (Fig. 2B). Moreover, immunoblot analysis of bulk lung lysates showed increased expression of both p16INK4A and CDKN1A/p21CIP1 in vehicle-treated BM mice compared with control mice, as expected. However, p16INK4A and p21CIP1 expression was reduced even below control in tamoxifen-treated BM mice (Fig. 2C). Expression of the tumor suppressor p19INKR, which can play a role in either senescence or apoptosis induction depending on the context, was found to be elevated in BM cells irrespective of tamoxifen treatment (Supplementary Fig. S2B).

Analysis of proliferation status by Ki67 staining showed occasional Ki67 cells in tumors of vehicle-treated BM mice, while the percentage of Ki67 cells increased strongly after tamoxifen treatment, in particular in BMK/KI mice (Fig. 2D). Increased apoptosis was observed in adenomas of tamoxifen-treated BM mice, but virtually no apoptotic cells were detected in vehicle-treated BMWT/KI mice (Supplementary Fig. S2C).

In conclusion, activation to MYC resulted in suppression of a number of senescence markers and elevated proliferative capacity in parts of BRAFV600E-induced tumors, correlating with accelerated tumor development.

**Suppression of p16INK4A and p21CIP1 by MYC starts early coinciding with accelerated tumor onset**

To assess whether MYC activation affected tumor initiation and senescence suppression already at an early stage, mice were vehicle treated or tamoxifen treated for a period of 7 or 14 days (i.e., 14 and 21 days after virus inhalation) and then sacrificed (Fig. 3A). H&E staining of lung sections showed that adenoma development had begun already at day 7 after tamoxifen treatment, in particular in BMKI/KI mice (Fig. 3A). In vehicle-treated BMWT/KI mice, tumors had started to appear at day 14, and the mice displayed lower lung weight as well as lower number and smaller size of tumors when compared with tamoxifen-treated BMKI/KI mice (Fig. 3A and Supplementary Fig. S3). Expression of both p16INK4A and p21CIP1 increased in vehicle treated in comparison with tamoxifen-treated mice, where expression of both proteins was suppressed to control levels already 7 days after tamoxifen treatment (Fig. 3B).

In conclusion, activation of MYC accelerated the onset of BRAFV600E-induced tumors already early after activation coinciding with suppression of BRAFV600E-induced expression of p16INK4A and p21CIP1.

**Suppression of p16INK4A and p21CIP1 requires continuous MYC activity and is linked to increased tumor size**

To investigate the consequence of inactivating MYC during tumor development, we exposed BMWT/KI mice to tamoxifen daily for a period of 7 days (MYC on), after which tamoxifen treatment was discontinued (MYC off) or continued until sacrifice due to disease (Fig. 3C). Surprisingly, lungs of MYC on/off mice displayed a similar number of tumors to mice under continuous tamoxifen treatment (Fig. 1B and Fig. 3C) and decreased survival compared with vehicle-treated BMWT/KI mice (data not shown). However, these tumors were of smaller size than those of continuously treated mice, resembling tumor sizes of vehicle-treated BM mice (Figs. 1B and 3C). Furthermore, the expression levels of p16INK4A and p21CIP1 were elevated in the lungs of MYC on/off mice compared with mice under continuous tamoxifen treatment (Fig. 3D). This suggests that suppression of p16INK4A and p21CIP1 requires continuous MYC activity, and that deactivating MYC restores BRAFV600E-induced senescence. This conclusion was further supported by the significant reduction in Ki67+ cells in tumors of MYC on/off mice compared with those treated daily with tamoxifen (Fig. 2D).

**Discussion**

Previous work from our laboratory and others highlighted a function for MYC in suppressing RAS/RAF-induced senescence in vitro (7, 8). However, whether MYC plays a role in overcoming this OIS barrier in vivo has not yet been determined. To address this question, we utilized a mouse model of BRAFV600E-induced lung adenomas, displaying high levels of
senescence-like growth arrest, by which their progression to malignant carcinoma is halted (5). These mice were intercrossed with mice containing a conditional \textit{MycER} allele encoding a tamoxifen-regulatable MycER protein (10). Our results show that MYC activation alone does not give rise to tumors within the 100 days of the experiment, but rather synergizes with BRAFV600E-induced tumorigenesis in a dose-dependent manner. This is evidenced by earlier tumor onset, increased numbers and sizes of tumors per lung, and shortened survival. Furthermore, this correlated with reduced expression of the senescence markers p16\textsuperscript{INK4A} and p21\textsuperscript{CIP1}, and H3K9me3 foci in tumor cells, and increased frequency of Ki67\textsuperscript{+} cells after MYC activation. These data are consistent with those of Juan and colleagues (12) investigating the impact of MYC activity on BRAFV600E-induced lung tumorigenesis. The increased tumor formation and suppression of p16\textsuperscript{INK4A} and p21\textsuperscript{CIP1} had started

**Figure 2.** MYC activation suppresses BRAF\textsuperscript{V600E}-induced senescence. A, analysis of p16\textsuperscript{INK4A} expression by IHC in whole lung sections of control (n = 2), vehicle-treated (VT) BM\textsuperscript{WT/KI} (n = 6), tamoxifen (TAM)-treated BM\textsuperscript{KI/WT} (n = 8), and BM\textsuperscript{KI/KI} (n = 3) mice. B, analysis of H3K9me3 and p16\textsuperscript{INK4A} (top two) expression by immunofluorescence using confocal microscopy as indicated. DAPI was used to stain nuclei. Bottom, colocalization of H3K9me3 and p16\textsuperscript{INK4A} staining. C, immunoblot analyses of whole lung lysates of p16\textsuperscript{INK4A} (top) and p21\textsuperscript{CIP1} (middle) in wild-type (lane 1); vehicle-treated BM\textsuperscript{WT/KI} (lanes 2 and 3); tamoxifen-treated BM\textsuperscript{KI/WT} (lanes 4 and 5); and BM\textsuperscript{KI/KI} (lanes 6 and 7). Tubulin (bottom) was used as a loading control. D, proliferation rates as assessed by Ki67 IHC staining of whole lung sections of BM\textsuperscript{WT/KI} + VT (n = 5), BM\textsuperscript{KI/WT} + tamoxifen (n = 4), and BM\textsuperscript{KI/KI} + tamoxifen (n = 4) mice. Quantitation of the results is presented in the bottom right panel. The analyses in A–D were done at endpoints when animals were sacrificed because of disease. Statistically significant differences from the control are shown. *, P < 0.05; **, P < 0.01.
already 7 days after MYC activation. In on/off experiments where MYC activation was discontinued after the first 7 days, we observed an increased number of tumors per lung area, as well as shortened survival compared with BRAFV600E alone. Interestingly, only small adenomas with regained expression of p16INK4A and p21CIP1 and strongly reduced Ki67 expression appeared, indicating that continuous MYC activity is required to maintain suppression of senescence. It is, however, not possible to draw conclusions from these data whether MYC not only blocks entry into the senescent state, but also forces cells to exit senescence - a topic that remains to be addressed in the future. Both p16INK4A and p21CIP1 are direct target genes repressed by MYC (7), and play a crucial role in blocking malignant progression of BRAFV600E-induced lung adenomas (5) as part of the Rb and p53 senescence pathways, respectively (6). Furthermore, these two pathways are frequently deregulated in human lung adenocarcinoma (1). Taken together, these results strongly suggest that suppression of the BRAFV600E-induced senescence-like state by MYC is an important threshold for continued lung tumor growth, thereby increasing the probability of additional oncogenic events. Furthermore,
inactivation of MYC in these lesions results in restored BRAFV600E-induced senescence.

Surprisingly, MYC did not promote adenoma to carcinoma transition in our model. In contrast, MYC either alone (13–15) or together with RAF1 (14) induced adenocarcinoma and metastasis in other lung tumor mouse models, however, with much longer latencies (around 60 weeks). Possibly, the massive lung adenoma burden in our model leading to early death through respiratory arrest precludes the onset of malignancy and metastasis. However, we cannot exclude that differences in expression of transgenes, identity of target cells, or functional properties of the different RAS/RAF-family proteins influence the outcome. Importantly, development of MYC-driven adenocarcinoma required additional genetic events to occur, such as activating mutations in KRAS, activation of the STAT pathway, and evasion of apoptosis (13–15). Taken together, these observations suggest that suppression of senescence by MYC is a discrete step in tumor development that precedes malignant transformation.

In conclusion, our results show that MYC plays an important synergistic role in lung tumorigenesis by suppressing a BRAFV600E-induced senescence-like state, resulting in increased tumor growth and reduced survival. This is in line with previous reports that MYC depletion induces senescence in vitro in human lung adenocarcinoma and malignant melanoma cells with activating mutations in KRAS, NRAS, or BRAF (8, 16), and highlights the importance of finding strategies for targeting MYC for cancer therapy, including the new concept of prosenescence therapy (6). Previous work using mouse tumor models with regulatable MYC or the dominant negative Omomyc has shown that inhibition of MYC often results in complete regression of tumors, frequently as a result of senescence induction (17, 18). In a Cre-activated KRASG12D-driven lung adenocarcinoma model, Omomyc caused tumor regression through increased apoptosis and senescence (17), which is similar to the outcome of MYC inactivation in our model, although we did not observe much apoptosis. The latter may be due to differences in tumor stage, activating oncogenes and/or the preserved endogenous MYC function in our model. In contrast, in a Tet-regulated combined KRASG12D/MYC mouse model where adenocarcinomas developed after longer latency, it was necessary to inactivate both KRAS and MYC to cause efficient tumor regression (15). This might be due to accumulation of additional mutations in these tumors as discussed above. Taken together, these findings emphasize the relevance of MYC targeting for lung cancer therapy. In the absence of drugs directly targeting MYC at present time, inhibitors of a number of upstream or downstream targets of the MYC pathway have been suggested, including BET and CDKs (3, 7). For instance, the senescence-suppression function of MYC is reversed by inhibition of CDK2 (7, 19). Such therapeutic strategies will be important to explore in the future, potentially in combination with MEK1 or RAF inhibitors, which has been shown to abrogate tumorigenesis in the BRAFV600E lung tumor model (5) and potently inhibit growth of human lung adenocarcinoma cells with BRAFV600E mutations. Furthermore, these drugs have recently shown promising results in clinical trials for patients with lung cancer carrying this mutation (20, 21).

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References


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