

Article

Nuclear Factor- κ B Signaling in Murine Models of Malignant Pleural Mesothelioma

Benteng Deng ^{1,2,†} , Ioanna Giopanou ^{3,†}, Lori Asarian ¹ , Ioannis Lilis ³, Jianlong Jia ¹, Marina Lianou ³, Dimitris Simoglou ³, Giannoula Ntaliarda ³ , Tonia A. Adamide ⁴, Isis E. Fernandez ^{1,2} and Georgios T. Stathopoulos ^{1,2,3,4,5,*} 

¹ Institute of Lung Health and Immunity (LHI), Comprehensive Pneumology Center (CPC), Helmholtz Zentrum München, German Center for Lung Research (DZL), 81377 Munich, Bavaria, Germany; benteng.deng@helmholtz-munich.de (B.D.)

² Department of Medicine V, LMU Klinikum, Ludwig-Maximilians-Universität München, 81377 Munich, Bavaria, Germany

³ Laboratory for Molecular Respiratory Carcinogenesis, Department of Physiology, Faculty of Medicine, University of Patras, 26504 Rio, Achaia, Greece

⁴ Department of Pulmonary Medicine, Nicosia General Hospital, Strovolos 2031, Nicosia, Cyprus

⁵ Medical School, University of Cyprus, Aglantzia 2029, Nicosia, Cyprus

* Correspondence: stathopoulos.georgios@ucy.ac.cy

† These authors contributed equally to this work.

Simple Summary

Malignant pleural mesothelioma (MPM) is an aggressive cancer linked to asbestos exposure. To shed light on the underlying mechanisms, we examined the roles of NF- κ B-regulatory enzymes IKK α and IKK β in three murine mesothelioma cell models. To this end, we targeted the activity of *Chuk* and *Ikbkb*, the two genes that encode the activity of IKK α and IKK β , respectively, using intrapleural and subcutaneous murine tumor models and a battery of in vitro functional assays. While decreasing the activity of both genes decreased growth-associated tumor metabolic activity, *Chuk* silencing led to more apoptosis, impaired colony formation and decreased tumor formation. Exploratory analyses of a publicly available human mesothelioma gene-expression dataset and our murine RNA-sequencing data complemented the in vitro data at the pathway and transcriptional levels. These findings confirm the importance of IKK/NF- κ B signaling complex in MPM and identify *Chuk* as a promising therapeutic target.

Abstract

Background: Malignant pleural mesothelioma (MPM) is an aggressive asbestos-associated cancer in which inflammatory pathways may contribute to tumor behavior. NF- κ B signaling is a central mediator of inflammation and cell survival, but the relative contributions of its associated enzymes, IKK α - and IKK β , in MPM models remain incompletely understood. **Methods:** We characterized the involvement of IKK α - and IKK β in tumor formation using several in vitro approaches and the intrapleural or subcutaneous delivery of three murine MPM cell models (AB2, AE17 and KPM1). The experimental approaches included shRNA silencing of *Chuk* and *Ikbkb*—the genes encoding the activity of IKK α - and IKK β —and tumor growth, apoptosis and colony formation measurements. Exploratory pathway and transcriptional analyses were carried out following RNA-sequencing of our murine cell lines and a publicly available human gene expression dataset was also analyzed to provide complementary evidence. **Results:** Activation of the IKK α - and IKK β was confirmed in the AB2, AE17 and KPM1 cell lines. Silencing both *Chuk* and *Ikbkb* decreased target and cross-target transcripts and metabolic growth. Importantly, *Chuk* silencing was associated



Academic Editor: Alfonso Baldi

Received: 10 August 2026

Revised: 10 September 2026

Accepted: 13 September 2026

Published: 16 September 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

with increased early apoptotic staining in AE17 and KPM1 cells, increased late apoptotic staining in AE17 cells and reduced colony formation in all cell models. Furthermore, *Chuk*-associated effects on tumor burden and activity were more notable than *Ikbkb*-associated effects in both intrapleural and subcutaneous tumor formation parameters. Finally, while canonical NF- κ B gene-set clustering in both human and murine tumor samples identified specific and shared pathways and transcriptional profiles supporting the in vitro murine assay findings, the analyses remained exploratory and did not specifically establish target dependency. Conclusions: Our data support the involvement of IKK/NF- κ B-associated signaling in cell survival, clonogenicity and tumor growth in several MPM murine cell models and identify *Chuk*-related signaling as a promising target in future studies.

Keywords: malignant pleural mesothelioma; NF- κ B; IKK α ; IKK β ; *Chuk*; *Ikbkb*; shRNA; RNA-seq; murine tumor models

1. Introduction

Malignant pleural mesothelioma (MPM), an uncommon but aggressive cancer that develops in the visceral or parietal lung pleura, is known to be associated with exposures to asbestos and related mineral fibers [1]. Its long latency and chronic inflammatory pathogenesis contribute to a substantial clinical burden [2]. In GLOBOCAN 2022 data, Fu and colleagues estimated 30,633 new mesothelioma cases and 25,371 deaths worldwide in 2022, with Europe accounting for nearly half of incident cases and marked regional and sex disparities [3]. Although this burden is much smaller than the almost 2.5 million lung cancer cases estimated globally in 2022 [4], MPM remains a major public health problem because of its poor prognosis and limited treatment options. Current management remains challenging despite platinum-pemetrexed chemotherapy and guideline-supported first-line immune-checkpoint blockade for selected patients with unresectable pleural mesothelioma [5–7]. Median survival has historically been reported at approximately 8–14 months from diagnosis, although outcomes vary by treatment setting and histological subtype [8]. Sarcomatoid or non-epithelioid tumors generally have less favorable outcomes than epithelioid tumors [9,10]. Molecular heterogeneity may also influence outcome and treatment response; genomic, transcriptomic, multi-omic, and pharmacogenomic studies therefore provide a rationale for biologically informed patient stratification and treatment development [11–15].

Nuclear factor- κ B (NF- κ B), a transcription factor implicated in tumors in various tissues [16–19], is activated by several inflammatory stimuli, such as cytokines, bacteria, viruses, radiation and mutated oncogenes. Its activation leads to genomic stress and alterations in cell-survival, immune and proliferative functions [20] that can trigger signaling via canonical and non-canonical pathways. Whereas canonical pathway triggers, such as infection and inflammatory signals, result in rapid activation of NF- κ B and its downstream target genes, non-canonical pathway signaling occurs over a longer timeframe and involves de novo protein synthesis and dimer formation. The identification of the I κ B inhibitory proteins and I κ B kinase (IKK) complexes provided important insights into the mechanistic and biological differences between the canonical and non-canonical pathways. Specifically, canonical signaling leads to the phosphorylation and activation of the NF- κ B essential modulator (NEMO) and IKK α :IKK β complex, and the subsequent release and nuclear translocation of RelA/p50 dimers [20]. On the other hand, non-canonical signaling initiated by upstream signaling converges on NF- κ B-inducing kinase (NIK)-dependent

IKK α activity, independent of NEMO and IKK β , and leads to p100 phosphorylation and subsequent nuclear signaling via RelB/p52 dimers [21–23].

Despite the 70% structural homology in their structural domains, important differences between IKK α and IKK β that may influence their role in health and disease have been identified [24,25]. While both are part of the canonical pathway and may be activated by innate and adaptive immune responses or exogenous mediators, such as TNF- α and LPS, IKK β 's kinase activity is 20–50-fold higher than that of IKK α [25,26], suggesting a more prominent role in the emergency, rapid immune responses. In addition to a role within the canonical signaling, however, IKK α can influence immune cellular processes not only via non-canonical NF- κ B pathways, but also via NF- κ B-independent pathways [23], giving it a broader arsenal in modulating cellular immunity. Finally, because mutations in genes encoding both IKK α and IKK β (*Chuk* and *Ikkkb*, respectively) have been associated with several malignancies and are involved in alterations in tumor microenvironment [27,28], here we examined both as part of our approach to identify the mechanisms underlying MPM.

Several inflammatory mediators that are potent activators of NF- κ B signaling pathways described above are released in response to asbestos-associated mesothelial injury and have been linked experimentally to the NF- κ B-dependent survival of damaged mesothelial cells [29]. Although previous studies have examined these inflammatory pathways and circulating biomarkers (e.g., RANTES, mesothelin-family proteins, ALDH1A3-associated STAT3/NF- κ B signaling, SOCS-1 signaling) in the context of MPM and how they may be affected by chemotherapy [30,31], the specific contributions of IKK α - and IKK β -linked signaling to MPM remain incompletely understood. To this end, we evaluated the phenotypic consequences associated with genetic manipulations of *Chuk* and *Ikkkb* in chemically induced or genetically engineered murine MPM models. We focused on IKK α and IKK β expression and localization, apoptosis and clonogenic assays and RNA-seq profiling in murine cell lines and complemented these findings with exploratory pathway analyses of a publicly available human dataset.

2. Materials and Methods

2.1. Mice

C57BL/6 (#000664) and BALB/c (#001026) mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). B6.129S4-*Kras*^{tm4Tyj/J} mice (hereafter *Kras*^{G12D}; #008179) [32] and B6.129P2-*Trp53*^{tm1Brn/J} mice (hereafter *Trp53*^{f/f}; #008462) [33] were also obtained from Jackson Laboratories, but subsequently bred on the C57BL/6 background at the University of Patras Center for Animal Models of Disease. Experiments were approved by the Prefecture of Western Greece's Veterinary Administration (approval 118018/578; 30 April 2014) and conducted according to Directive 2010/63/EU. Mice were matched for sex, weight (20–25 g), and age (6–12 weeks) prior to experimental manipulations.

2.2. Cell Lines, Culture Conditions, and shRNA Constructs

BALB/c mouse AB2 and C57BL/6 mouse AE17 MPM cells were a gift from Dr. YC Gary Lee (University of Western Australia, Perth, Australia) [34]. C57BL/6 mouse KPM1 cells, primary WT pleural mesothelial cells (PMCs) and urethane-induced lung adenocarcinoma (CULA) *Trp53*^{f/f} allele cells were generated in our laboratory, using previously described protocols for the KPM1 line [35], for PMC [35] and by using a Cre-recombinase conditional approach [36], respectively. Public repository identifiers for the cell lines were as follows: CVCL_LJ79, CVCL_4408 and CVCL_A9KU, for AB2, AE17 and CULA, respectively. The KPM1 line is deposited at the Laboratory for Molecular Respiratory Carcinogenesis cell-line facility (Patras, Greece) and is available upon request. Because they have been

previously characterized [17,35,37], PMC and CULA cells were only used as controls for the verification of the *Kras* or *Trp53* mutation status of the AE17, AB2 and KPM1 cell lines. The human cell lines MET5A, MSTO-211H, and ZL34 (public repository identifiers: CVCL_3749, CVCL_1430, and CVCL_5906, respectively) were only used as controls for NF- κ B-subunit expression analysis. All cell lines tested negative for mycoplasma.

Human cell lines were cultured at 37 °C in 5% CO₂ in DMEM (MET5A and ZL34) or RPMI 1640 (MSTO-211H) and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM pyruvate, 100 U/mL penicillin, and 100 µg/mL streptomycin. Murine pleural mesothelial cells (AE17, AB2 and KPM1) were cultured in DMEM as described above for the human cell lines and then used following shRNA transfection for the further characterization of MPM tumor cell models. For stable lentiviral shRNA transfection, 10⁵ tumor cells were treated with lentiviral shRNA particles according to the manufacturer's protocol (Santa Cruz Biotechnology, Dallas, TX, USA). For *Chuk* and *Ikbkb*, a pool of three target-specific sequences was used, whereas a proprietary, scrambled sequence (shC) was used as a control (Supplementary Table S1). GFP was incorporated in the vectors and was induced upon successful transduction. Non-transfected cells were then selected using puromycin (2–10 µg/mL; Gibco, Invitrogen, Carlsbad, CA, USA). For the characterization of tumor models, cells were harvested, stained with trypan blue and counted using a hemocytometer. Cell suspensions with >95% viability were then used for in vitro studies or administered to mice, as described previously [35].

2.3. Metabolic Activity, Colony Formation, and Apoptosis Assays

Growth-associated metabolic activity of the various cell lines was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. Following shRNA transfection, cell lines (AB2, AE17 and KPM1) were seeded (3×10^3 cells per well in 96-well plates, $n = 6$ per shRNA transcript) and cultured for four days. Each day, 10 µL of MTT (Sigma, St. Louis, MO, USA) working solution (5 mM MTT in PBS) was added to each well. Four hours later, 100 µL acidified isopropanol was added, followed by sediment solubilization. Absorbance (492 nm) was measured in a MR-96A microplate reader (Mindray, Shenzhen, China). Absorbances of wells with DMEM alone, in the absence of cells, were used as background and were subtracted from the readings of experimental well. Corrected absorbances were subsequently used for statistical analyses.

For colony formation assays, cells were seeded in 6-well plates (100 cells/well for AB2 and AE17 cell lines, $n = 6$ and 4, respectively; 300 cell/well for KPM1, $n = 3$) were closely monitored for 2 weeks until colony formation. Colonies were then fixed and stained with 0.5 g Crystal Violet (0.05% *w/v*) (TargetMol, Boston, MA, USA) for 20 min at room temperature and counted.

Detection of early and late apoptosis was done using an annexin-V-7AAD assay. Briefly, 10⁶ cells in 100 µL Annexin-V 1x buffer were incubated at room temperature with Annexin-V (ImmunoTools, Friesoythe, Germany; 31490014, 1:200 dilution) and 7AAD (eBioscience Inc., San Diego, CA, USA; E00031-1632, 1:20 dilution), for 15 min. Late apoptotic cells were characterized as double positive Annexin-V/7AAD, while early apoptotic cells as represented by Annexin V+/7-AAD- labels. Statistical analyses were done on a set of three replicates per cell model. Cell counts were carried out using CyFlow ML (Partec, Darmstadt, Germany) and analyses with FlowJo software v10.10.1 (FlowJo, Ashland, OR, USA).

2.4. PCR and RNA Sequencing

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and the RNeasy kit (Qiagen, Hilden, Germany) and reverse transcribed using SuperScript III (Invitrogen, Carlsbad, CA, USA). To identify the *Trp53* mutation status in PMC, AE17, AB2, KPM1 and CULA cells, RT-PCR was performed using SYBR Green Master Mix on a StepOnePlus instrument (Applied Biosystems, Carlsbad, CA, USA). Following shRNA transfection, the extent of gene silencing was reported following quantitative RT-PCR (qPCR). The absence of mycoplasma in the cell culture experiments was ensured with a standard PCR reaction. All primers are listed in Supplementary Table S3. Primers were used according to previously published protocols to ensure maximum efficiency [38,39]. For the qPCR, relative expression was normalized to *Gusb* and calculated against the indicated control condition using the $2^{-\Delta C_t}$ method [40].

For RNA-seq, total RNA from shRNA-targeted AB2, AE17 and KPM1 cell lines was extracted from 106 cultured cells. Libraries were prepared from 2 µg of total RNA and single-end sequencing was carried out on Illumina Hiseq 4000 (San Diego, CA, USA). FASTQ files were aligned to GRCm38/mm10 using STAR v2.7, and read counts were generated with featureCounts/Subread before downstream differential-expression analysis in R v4.4.2 using limma v4.22.0 and edgeR v4.4.0 [41,42]. Genes with a nominal $p < 0.05$ and an absolute log2 fold change of ≥ 1.7 were retained for the exploratory summaries. Differentially expressed genes (DEGs) in the 3 cell lines were then analyzed for covariance among the different shRNA targets (control, *Chuk*, *Ikbkb*) and column z scores were used for hierarchical clustering. Heatmaps and PCA plots were generated in R using gplots (v3.2.0)/heatmap.2 and factoextra (v2.0.0)/fviz, respectively. Raw BAM files and processed data are deposited in the NCBI Gene Expression Omnibus under accession GSE129984 [43].

2.5. Intrapleural and Subcutaneous Tumor Models

For intrapleural tumor experiments, 2×10^5 AE17 or KPM1 cells transfected with shRNA (control, *Chuk* or *Ikbkb*) were delivered in 100 µL PBS to isoflurane-anesthetized C57BL/6 mice. Similarly treated AB2 cells (1.5×10^5) were administered to BALB/c mice. The number of mice used for each cell line and shRNA construct is listed in Supplementary Table S4. Following the administration of cell suspensions, mice were monitored during post-surgical recovery, and daily thereafter, and were sacrificed when moribund. Survival time was recorded and pleural fluid and tumors were collected. The isolated malignant pleural effusion (MPE) fluid was diluted ten-fold in red blood cells lysis buffer (155 mM NH₄Cl, 12 mM NaHCO₃, 0.1 mM EDTA). Total nucleated pleural cells were counted using a hemocytometer and cytopsin preparations were then generated using 5×10^4 cells per slide for further cytological analysis. Lungs, visceral pleural tumors, parietal pleural tumors, and chest walls were fixed in 4% paraformaldehyde overnight, and tumor burden was subsequently assessed using tumor weight.

For subcutaneous solid tumor experiments, 10^6 cells transfected as described above were injected in the rear flanks of either C57BL/6 (KPM1 or AE17 cells) or BALB/c mice (AB2 cells). The number of mice used for each cell line and shRNA construct is listed in Supplementary Table S4. Once a week for 4 weeks, three orthogonal tumor dimensions (δ_1 , δ_2 , δ_3) were performed per mouse, and tumor volumes were calculated as $\pi \cdot \delta_1 \cdot \delta_2 \cdot \delta_3 / 6$ [44,45].

2.6. Histology, Immunohistochemistry, Immunofluorescence, and Immunoblotting

Whole lungs were fixed overnight in 4% paraformaldehyde and then embedded in paraffin. All primary and secondary antibodies and detection methods are listed in Supplementary Table S2.

For immunohistochemistry, 4- μ m paraffin sections were deparaffinized, rehydrated, subjected to heat-induced epitope retrieval in 10 mM sodium citrate (pH 6.0), and blocked with 3% H₂O₂ and then 3% bovine serum albumin for endogenous peroxidase quenching and against nonspecific antibody binding, respectively. Anti-IKK α (sc-7606, rabbit polyclonal IgG (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-IKK β (MA5-16154, mouse monoclonal IgG, 1:100, Invitrogen, Carlsbad, CA, USA) primary antibodies were used in an overnight incubation at 4 °C. Horseradish peroxidase (HRP) was used as the detection method, according to the manufacturer's instructions (EnVision; Dako, Glostrup, Denmark). Sections were counterstained with Harris's hematoxylin, dehydrated, mounted and imaged using an Axio Lab.A1 microscope with an AxioCam ERc 5s camera (Zeiss, Jena, Germany).

For immunofluorescence, cells were fixed overnight in 4% paraformaldehyde at 4 °C and then incubated with primary and secondary antibodies (Invitrogen, Carlsbad, CA, USA). Cells were counterstained with Hoechst 33258 (Sigma-Aldrich, St. Louis, MO, USA) and mounted with Mowiol 4-88 (Calbiochem, Gibbstown, NJ, USA). Primary antibody was omitted for the negative control. Imaging was carried out using an AxioObserver.D1 inverted microscope with an AxioCam ERc 5s camera (Zeiss, Jena, Germany), and image processing was done using Fiji v1.54p [46].

Nuclear and cytoplasmic extracts were prepared using the NE-PER Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA), separated on 12% SDS polyacrylamide gels using electrophoresis (PAGE), transferred to polyvinylidene difluoride membranes (PVDF, Merck Millipore, Darmstadt, Germany), probed with specific antibodies and then visualized after incubation with enhanced chemiluminescence substrate (Merck Millipore, Darmstadt, Germany).

2.7. Public Human Expression Data and Pathway Gene Sets

Human gene expression analysis (Affymetrix Human Genome U133 Plus 2.0 Array platform) was carried out on a publicly available dataset (GEO accession GSE51024), which included 55 MPM tumor samples and 41 paired non-tumor lung parenchyma samples [47]. Pathway Interaction Database (PID) [48] analyses used canonical and atypical NF- κ B pathway gene lists. The atypical NF- κ B gene list specifically identified with *Ikkbb* signaling-related genes. Unsupervised clustering of individual patients was performed using a hierarchical algorithm in the R package cluster v.2.1.6 and the expression level of the genes in the canonical or atypical lists.

2.8. Statistics

Unless otherwise stated, plotted data are mean $\pm$ SD or mean $\pm$ 95% confidence interval, as specified in the corresponding figure legend. All data were normally distributed and were analyzed with one- or two-way ANOVA followed by Bonferroni post hoc tests, as appropriate. Kaplan–Meier survival estimates were performed with log-rank tests. Data acquisition was carried out on blinded samples. *p*-values were two-tailed. *p* < 0.05 was considered significant. Statistical analyses and graphs were done on Prism v5.0 (GraphPad, La Jolla, CA, USA).

3. Results

3.1. Characterization of MPM Models and NF- κ B-Associated Expression Patterns

Because our previous findings highlighted the importance of *Kras* and *Trp53* deletions in mice fostering malignant pleural mesothelioma [35], and because the *Trp53* mutation status is unknown in the AE17, AB2 and KPM1 cell models, we first described the origin and genotypes of these cell lines, as they will be used throughout this study (Figure 1A).

Primary mesothelial cells (PMCs) and urethane-induced lung adenocarcinoma (CULA) cells were used as positive controls for presence of *Kras* and *Trp53* in our RT-PCR assays. Our results show that AB2 and AE17 cells were WT for *Trp53* mutations, whereas KPM1 cells showed a specific deletion (Figure 1B). We next examined the NF- κ B-associated expression and localization patterns in both our murine cell lines (AB2, AE17 and KPM1) as well as three human MPM cell lines (MSTO211H, ZL34 and MET5A). We found that the p52 subunit was detected in both the cytoplasmic and nuclear fractions of the non-malignant MET5A cells, whereas MSTO211H cells expressed RelB in both cell compartments and ZL34 cells expressed both RelA and RelB. In the ZL34 cells, RelB appeared to be expressed higher in the nucleus and RelA overexpressed in cytoplasm (Figure 1C). The three murine cell lines AB2, AE17 and KPM1 had a similar pattern of highly expressing RelB and p52 in both the cytoplasm and the nucleus. This is consistent with a possible non-canonical NF- κ B-associated pattern in both human and murine cell models. These results led us to then investigate the expression pattern for the upstream NF- κ B-activating kinases (IKK α and IKK β) in MPM. Using immunohistochemistry and samples from BALB/c and C57BL/6 mice that developed MPM upon intrapleural injection of AB2, AE17 or KPM1 cells, we identified distinct, upregulated immunoreactivity for IKK α and IKK β (Figure 1D). In particular, in MPM developed from AE17 intrapleural cell delivery in mice, IKK α had slightly higher expression in the cytoplasm, whereas in MPM developed by AB2 intrapleural cells delivery in mice, both IKK α and IKK β showed increased immunoreactivity at the tumor site. Finally, after KPM1 intrapleural delivery, both subunits were overexpressed, but IKK β expression was slightly higher. Together with the subunit localization results from the Western blot studies, these findings suggest that nuclear RelB and p52 localization and possible engagement of NF- κ B-associated signaling may characterize these models.

3.2. Effects of IKK α and IKK β Gene Silencing on Apoptosis of MPM Cells

To test the impact of IKK expression in tumor cell proliferation and survival we used gene silencing of *Chuk* and *Ikbkb*, the genes encoding IKK α and IKK β expression, respectively, using lentiviral shRNAs in all three murine cell lines (AB2, AE17 and KPM1). The shRNA constructs are described in Supplementary Table S1. The extent of gene silencing in each cell line was validated with quantitative PCR (qPCR) using specific primers (Figure 2A; Supplementary Table S3) that detected *Chuk* and *Ikbkb* mRNA. GFP expression was present in all cell lines, thus validating the vector transfection (Figure 2B). We further validated IKK α and IKK β expression following transfection using immunofluorescence (Figure 2C). Consistent with our qPCR results, we observed some cross-silencing of *Chuk* when *Ikbkb* shRNA was used, especially in the AE17 and KPM1 cell lines. It is not clear whether this was due to particular cell line characteristics or whether it was a compensatory response, given the similarities between IKK α and IKK β . We next investigated how the decrease in *Chuk* and *Ikbkb* gene expression impacted the survival of the malignant cells using an early and late apoptotic cell death assay (Figure 3). There was no effect of *Ikbkb* gene silencing on cell survival or of *Chuk* gene silencing in AB2 cells. In contrast, *Chuk* gene silencing revealed statistically significant increases in early apoptosis in AE17 and KPM1 cell lines (Figure 3B), as well as increased late apoptosis in AE17 cells (Figure 3A). Whether these effects of *Chuk* silencing on apoptosis differed due to differences in *Kras* or *Trp53* mutation between the cell lines or because of the slight extent of cross-silencing between *Chuk* and *Ikbkb* remains an open question.

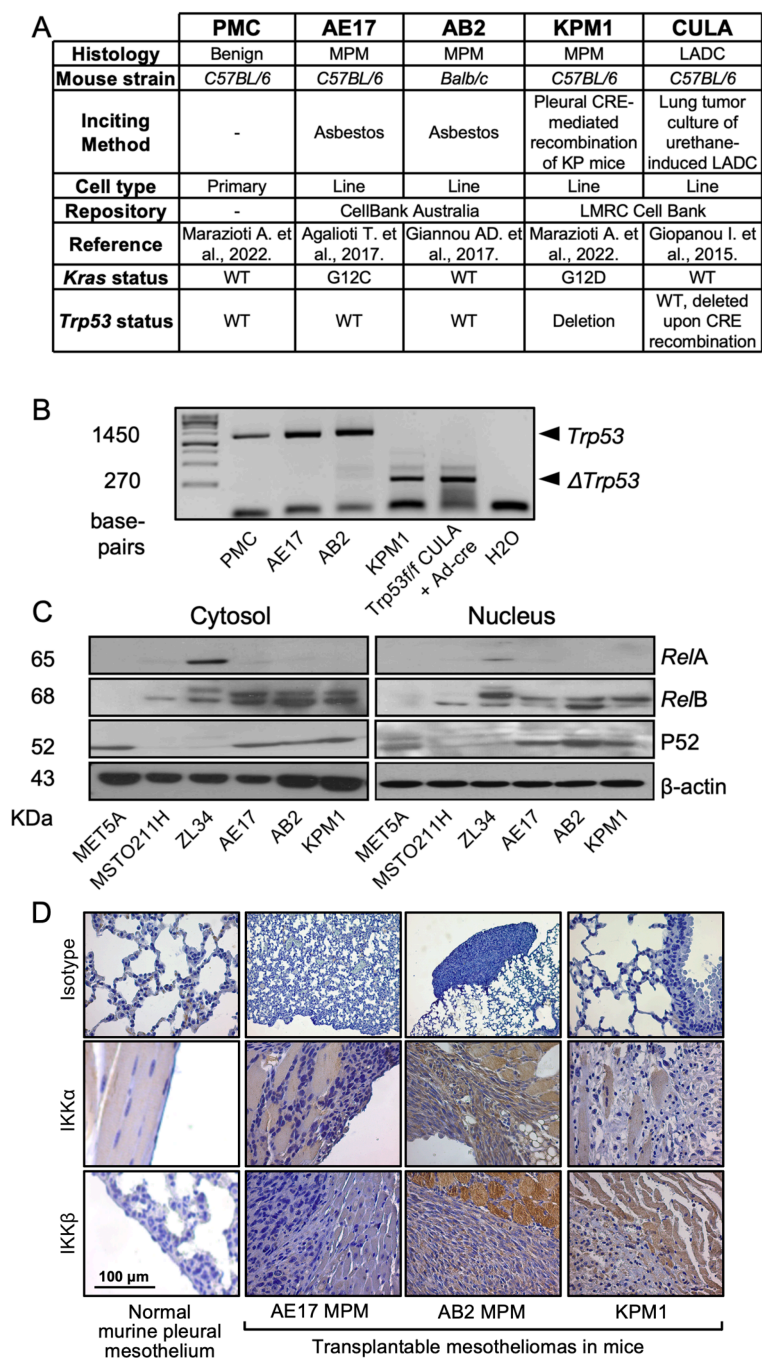


Figure 1. Characterization of MPM models and NF- κ B-associated protein patterns. Summary of histology, origin, induction method, source/repository, reference, and *Kras*/*Trp53* status for primary mesothelial cells (PMCs), AE17, AB2, KPM1, and CULA cell lines used in the current experiments (A) [17,35,37,45]. Quantitative PCR showed the *Trp53*^{flf} deletion-associated 270 bp band was present in KPM1, but not AE17 and AB2 cell models. Ad-Cre-treated *Trp53*^{flf} CULA cells were used as a positive control (Supplementary Materials Figure S08) (B) [36]. Western blot analyses of RelA, RelB, and p52 in cytoplasmic and nuclear extracts from human MET5A, MSTO-211H, and ZL34 cells and murine AE17, AB2, and KPM1 cells. β -actin is shown as a loading control (Supplementary Materials Figures S01–S07) (C). Representative IKK α and IKK β immunohistochemistry images from normal murine pleural mesothelium and tumors derived from intrapleural AE17-, AB2-, or KPM1-administration (D). The negative-control condition omitted primary antibody and is therefore described as a non-primary control. Scale bar, 100 μ m.

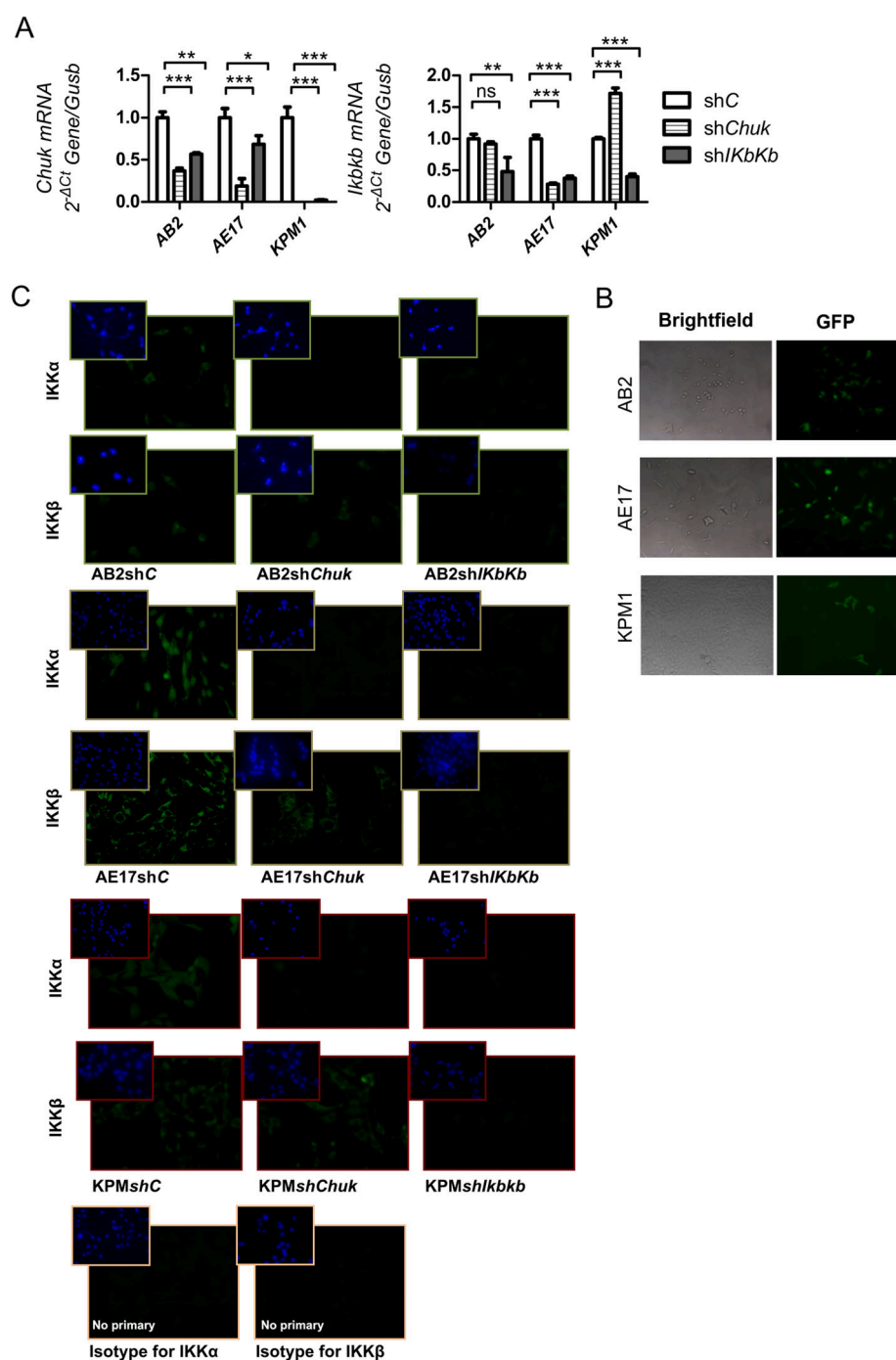


Figure 2. Validation of lentiviral shRNA silencing of *Chuk* and *Ikbkb* in murine MPM cell lines. Representative qPCR analysis showing expression of *Chuk* (encoding IKKα) and *Ikbkb* (encoding IKKβ) relative to *Gusb* mRNA (A). Representative GFP images validating vector expression/transfection. The GFP expression was the result of a built-in construct in each of the lentiviral probes. Brightfield pictures of the cell cultures (left) are provided for reference (B). Representative IKKα and IKKβ immunofluorescence images (green) with Hoechst 33258 nuclear counterstain (blue) following lentiviral transfection. Negative-control (bottom two panels) conditions omitted primary antibody. Lack of green fluorescence denotes absence of IKKα and IKKβ protein (C). Data in (A) are mean $\pm$ 95% confidence intervals. Significances (** $p < 0.01$, *** $p < 0.001$, * $p < 0.05$ and ns $p > 0.05$) were obtained after two-way ANOVA with Bonferroni post hoc tests.

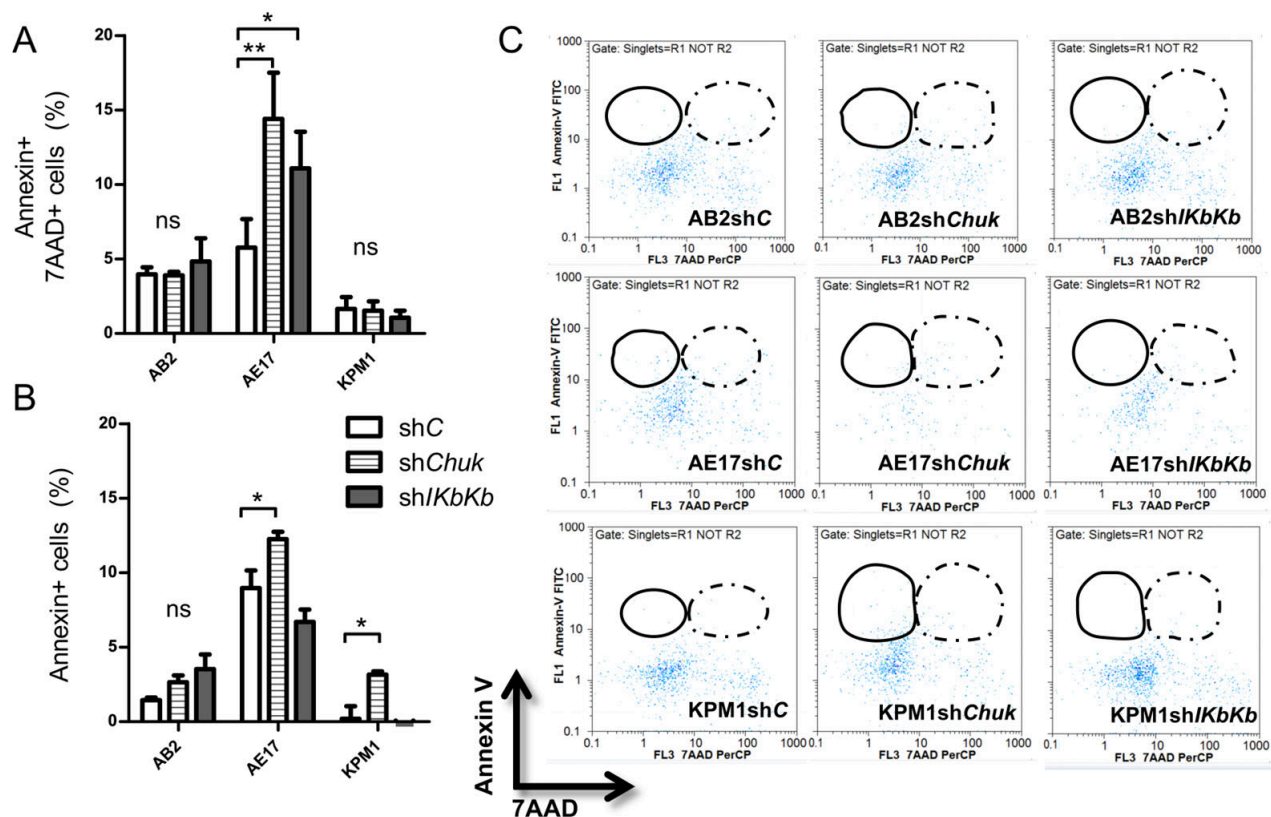


Figure 3. Effects of *Chuk* and *Ikbkb* silencing on apoptosis of MPM murine cell models. Apoptosis in *Chuk*- and *Ikbkb*-silenced cells was assessed by flow cytometric determination of annexin V and 7AAD staining. Annexin and 7AAD double positive cells denote late apoptosis (A), whereas AnnexinV+/7AAD- cells denote early apoptosis (B). Representative dot-plots and gating strategy from the experiments described in (A,B) are shown in (C), with solid and dashed circles denoting early- and late-apoptotic cells, respectively. Data are mean $\pm$ SD. Significances (** $p < 0.01$, * $p < 0.05$ and ns $p > 0.05$) were obtained after two-way ANOVA with Bonferroni post hoc tests.

3.3. Effects of *Chuk* and *Ikbkb* Silencing on Tumor Cell Proliferation and Clonogenicity

We next investigated whether silencing $IKK\alpha$ and $IKK\beta$ gene expression had a differential impact on cell proliferation, as measured with a metabolic activity (MTT) assay, and on clonogenicity among our MPM cell models (Figure 4). Across all models, *Chuk* gene silencing was associated with reduced MTT signal relative to control, indicating reduced growth-associated metabolic activity. This measure has generally been well-correlated with a direct cell-count measure of proliferation in previous studies, but it is not a measure of cell number, per se. The reduction in MTT signal by *Chuk* silencing in AB2 cells, which are WT for both *Kras* and *Trp53*, (Figure 4B) and the lack of apoptosis (Figure 3) suggests that reduced metabolic growth and apoptosis may be mediated by different mechanisms. Silencing *Ikbkb*, on the other hand, significantly decreased the MTT signal only in the KPM1 cell model. Finally, colony formation was significantly decreased by *Chuk* signaling in all cell models, whereas *Ikbkb* silencing had no effect. These results suggest that, at least under our experimental conditions, *Chuk* signaling and, therefore, $IKK\alpha$, has a significant impact on proliferation and colony formation in MPM cell models.

3.4. Effects of *Chuk* and *Ikbkb* Silencing on MPM Tumor Growth In Vivo

To further clarify the importance of IKK α and IKK β in tumor growth associated with MPM, we next used the three shRNA-transduced murine cell models characterized above and monitored MPM progression following their instillation via either an intrapleural or a subcutaneous route. Survival was monitored and measurements of the development in tumor weight, pleural fluid accumulation and number of nucleated cells in pleural fluid were done as previously described [35]. Mice showed worse survival when injected with malignant cells in which IKK α and IKK β expression were inhibited, with IKK α -inhibition being more effective. This is consistent with our in vitro findings showing that IKK α loss reduced cell proliferation ability and enhanced cancer cell death (Figure 5).

Following delivery of AB2 cells, which are WT for both *Kras* and *Trp53*, tumor size and pleural fluid formation decreased significantly regardless of whether the AB2 cells were transduced with *Chuk* or *Ikbkb* shRNA, whereas the number of nucleated cells in pleural fluid was significantly decreased only when *Chuk* was inhibited (Figure 5A). Similar results were seen in tumors derived from AE17 cells, characterized by *Kras*^{G12C} mutation (Figure 5B). Finally, KPM1-derived tumors showed significant decreases in growth parameters only when *Chuk*, but not *Ikbkb* was silenced, highlighting the involvement of IKK α signaling in tumors characterized by *Kras*^{G12D} mutation and *Trp53* loss of action (Figure 5C).

Finally, subcutaneous ectopic tumor-growth measurements confirmed the pleural tumor characteristics. Whereas both IKK α and IKK β were important in tumors derived from AB2 and AE17 cells, only IKK α led to a statistically significant decrease in KPM1-derived tumors, further supporting the IKK α involvement in tumors characterized by *Kras*^{G12D} mutations and *Trp53* deletions (Figure 5C).

3.5. NF- κ B-Signaling Gene Expression Is Associated with MPM in Human Tumors

We next assessed whether NF- κ B pathway-related genes were expressed in human MPM tumors. We chose two gene lists that were part of the Pathway Interaction Database [48] and that focused either on a combination of *Chuk* and *Ikbkb* (23 genes; canonical pathway) signaling or only on *Ikbkb* signaling alone (17 genes; referred to as atypical, but resembling the non-canonical pathway) (Figure 6A). Expression of these genes was identified in a publicly available data set (GEO Datasets accession ID: GSE51024) containing 55 MPM and 41 paired non-tumor lung parenchyma tissue samples from patients with MPM [47,48]. Unsupervised clustering, performed on the gene expression values of all samples, revealed that the canonical and atypical pathways resolved the samples into four and three clusters, respectively (Figure 6B). A follow-up analysis based on the number of non-tumor and tumor samples per cluster, showed that cluster 2 within both the canonical and the atypical pathways contained more tumor samples than the remaining clusters and characterized a subgroup of samples based on their gene expression. This suggests that, at least for some MPM patients, both NF- κ B signaling pathways may be involved in MPM tumor formation. While this remains an exploratory association, similar analyses identifying other genes present in such sample clusters and whether they may correlate with disease progression of tumor mutation could provide further information about the mechanistic contribution of IKK α and IKK β signaling in some MPM patients.

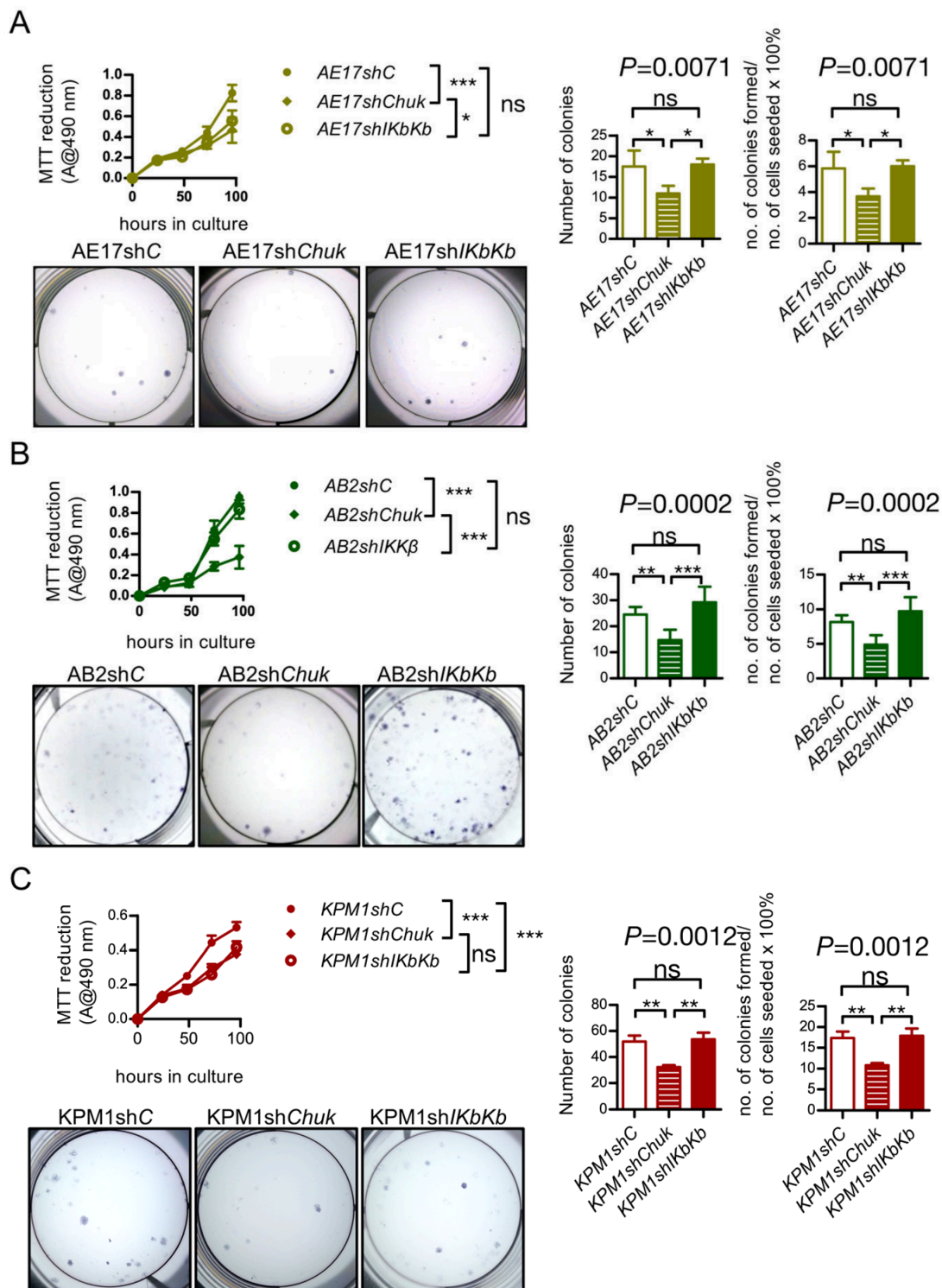


Figure 4. Effects of *Chuk* and *Ikbkb* silencing on tumor cell proliferation and clonogenicity. A metabolic growth assay (MTT) was used to identify changes in cell proliferation, and standard clonogenicity assays were used to determine colony formation in *Chuk*- and *Ikbkb*-silenced AE17 (A), AB2 (B) and KPM1 (C) cells. Data are expressed as mean $\pm$ 95% confidence intervals. Significances (** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ and ns $p > 0.05$) were obtained following one- or two-way ANOVA with Bonferroni post hoc tests for MTT and colony formation assay, respectively.

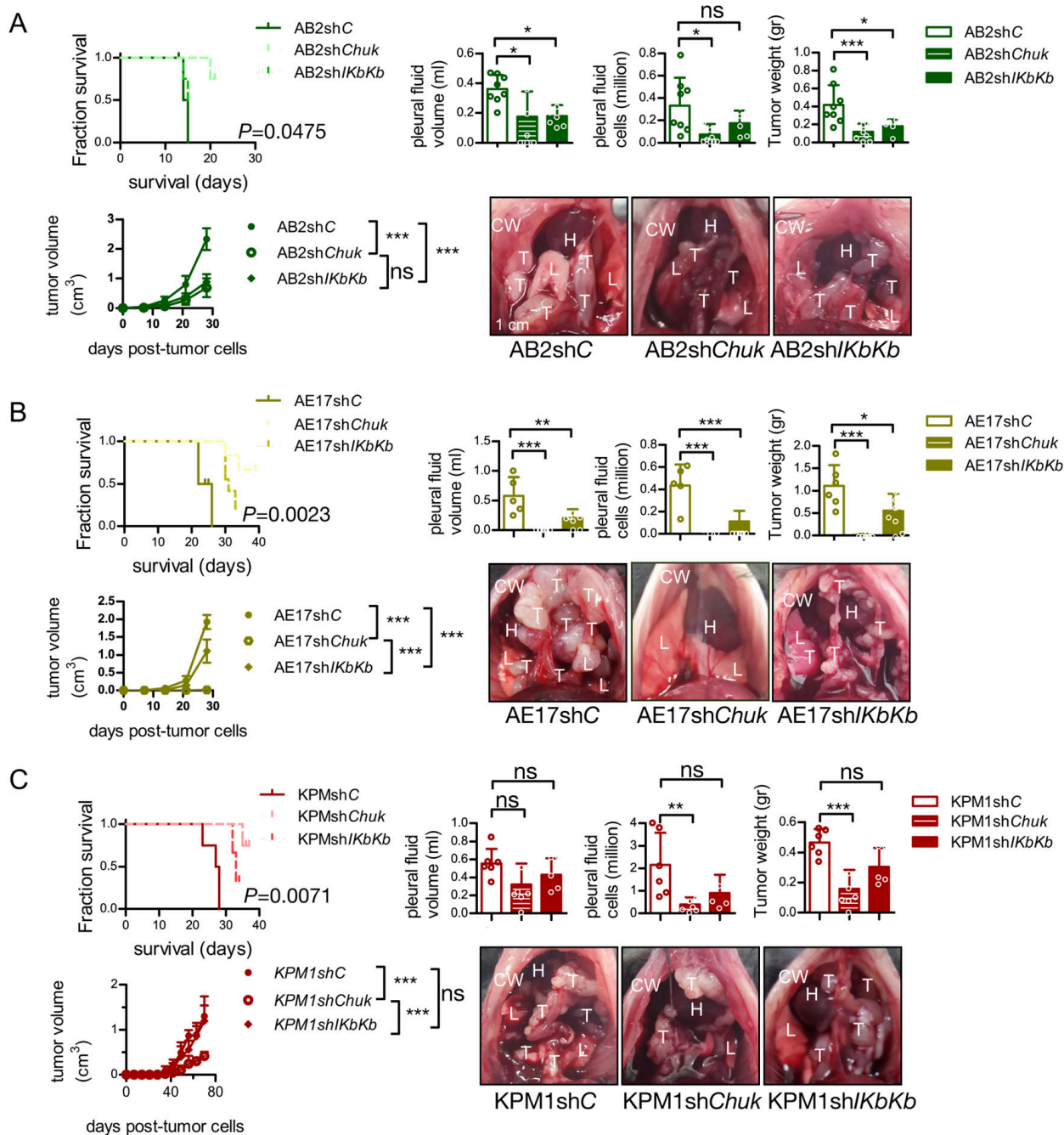


Figure 5. *Chuk* and *Ikbkb* silencing affects in vivo tumor development. *Chuk*- and *Ikbkb*-silenced MPM cell lines were delivered either intrapleurally or subcutaneously in BALB/c or C57BL/6 mice, as described in the method. The AB2, AE17 and KPM1 cell lines are shown in (A), (B), and (C), respectively. For each line, the survival curves are shown on the top left, and the tumor volumes of the subcutaneous tumors on the bottom left. The right-side panels show the results after intrapleural delivery of the MPM cells, with pleural malignancy parameters on the top right and representative pictures (T, tumors; L, lungs; CW, chest wall; H, heart) on the bottom right. The mouse group sizes are reported in Supplementary Table S4. Data are expressed as mean $\pm$ 95% confidence interval. Significances (** $p < 0.01$, * $p < 0.05$ and ns $p > 0.05$) were obtained after log-rank (Mantel–Cox) Tests for comparison of survival curves and one- or two-way ANOVA with Bonferroni post hoc tests for subcutaneous or intrapleural tumor parameters, respectively.

3.6. Transcriptional Responses Associated with *Chuk*- and *Ikbkb*-Silencing in Murine MPM Models

RNA sequencing of *Chuk*- or *Ikbkb*-silenced AB2, AE17 and KPM1 cells revealed that 188 genes were differentially expressed (vs. control transfections; $p < 0.05$); 76 and 49 genes were upregulated or downregulated, respectively, when both *Chuk* and *Ikbkb* were silenced. Specific to each deletion, 28 and 66, and 86 and 74 genes were up- or down-regulated for *Chuk* or *Ikbkb*, respectively (Figure 7A). The clustering of the 188 DEGs specifically related to *Chuk*, *Ikbkb* of control transfections for each of the MPM cell lines (AB2, AE17 and KPM1) are shown as summaries in PCA biplots and heatmap (Figure 7B,C) and indicate that specific genes are associated with each experimental condition/cell line. These exploratory analyses identify specific sets of genes that may characterize the development of murine MPM and could be used as a starting point in identifying NF- κ B-signaling-related therapeutic targets to be tested in future studies.

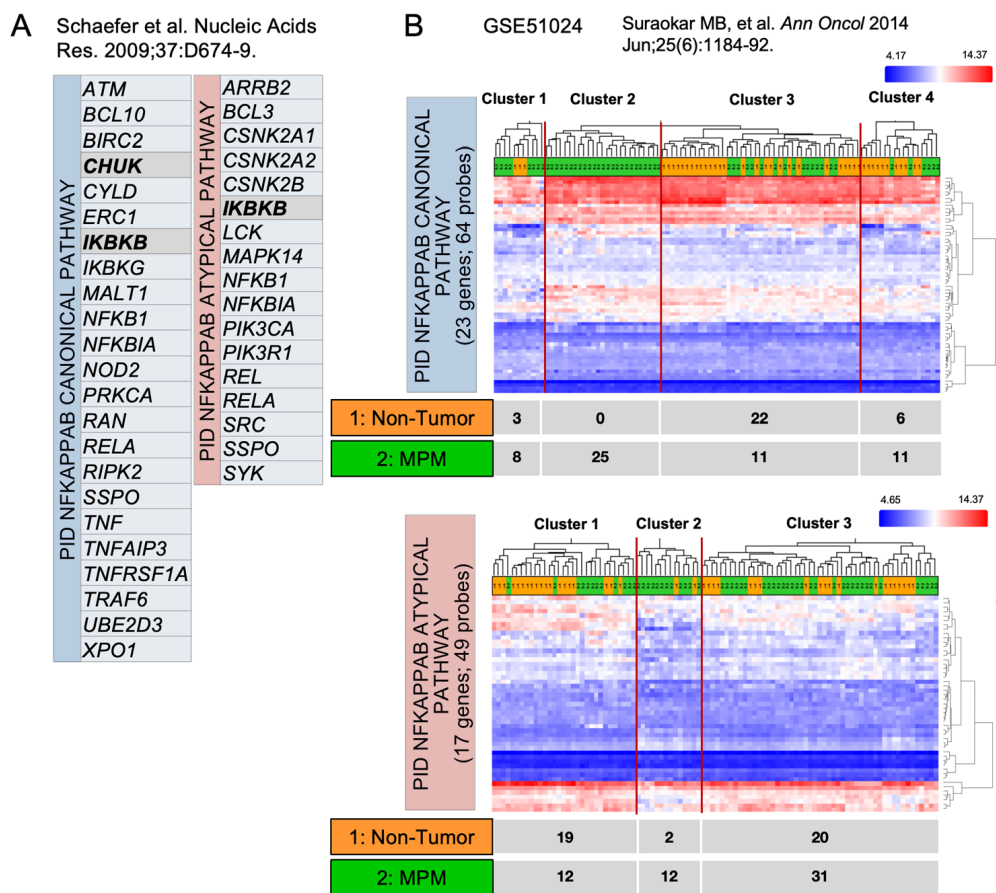


Figure 6. NF- κ B gene expression pathways are represented in discrete clusters of human MPM. Two gene lists of 23 and 17 genes that represented the NF- κ B canonical and atypical signaling pathways, respectively, ref. [48], were used as input for the analyses of human non-tumor and malignant pleural mesothelioma samples (A). Unsupervised hierarchical clustering of the gene expression in non-tumor and tumor samples revealed that the distribution of sample types in cluster 2 were significantly different than the other clusters when using either canonical or atypical gene sets as clustering input. The number of samples (non-tumor in green and tumor in orange) are listed under the heatmaps and were used in the subsequent analyses after the hierarchical clustering (B) [47]. Significances between clusters were obtained following χ^2 and corrected Fisher-exact follow-up tests, p 's < 0.001 , for canonical and atypical pathways respectively).

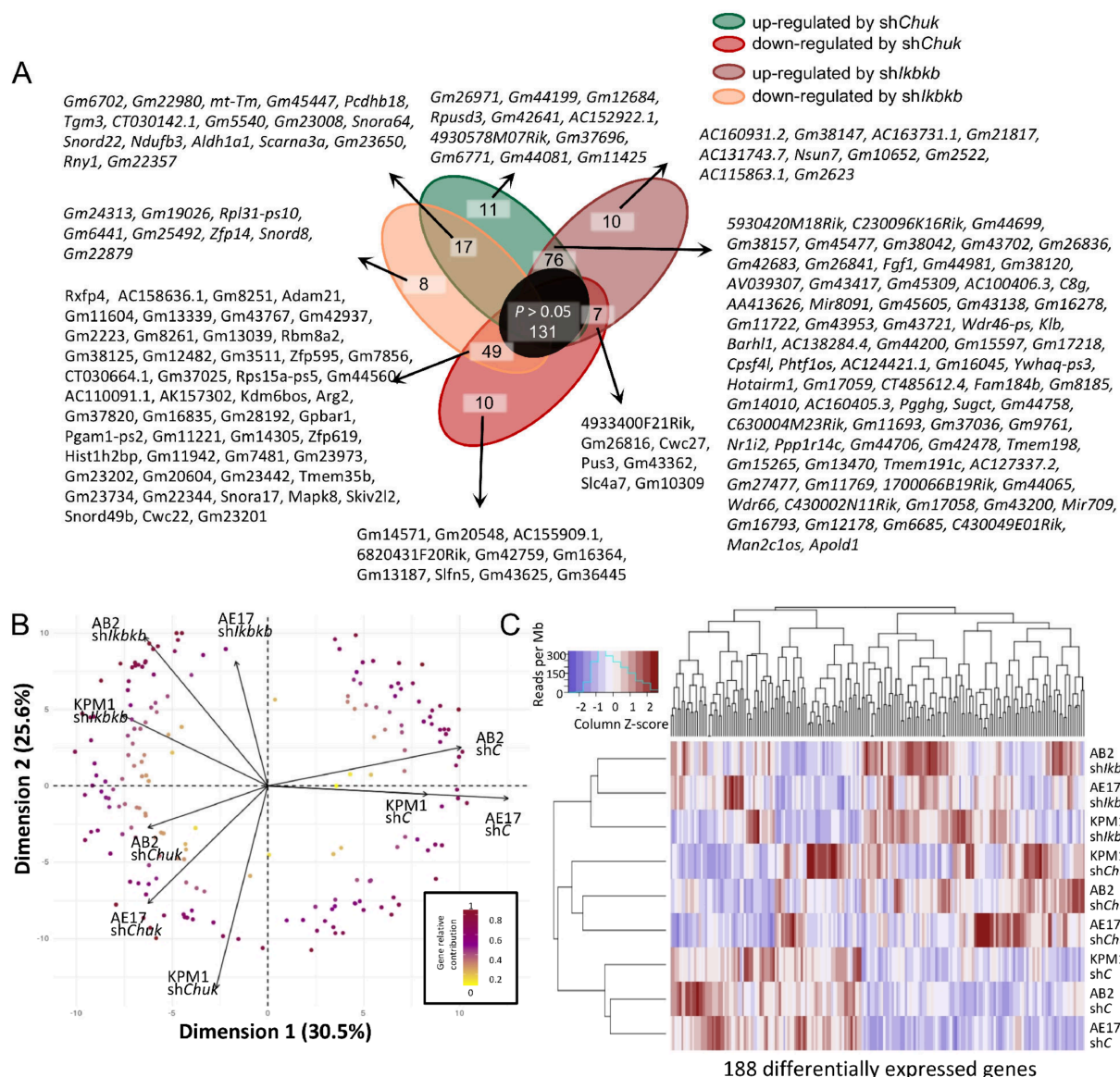


Figure 7. Exploratory transcriptional responses associated with *Chuk* and *Ikbkb* gene silencing in AB2, AE17 and KPM1 cell models. Venn diagram identifying differentially expressed genes, up- or down-regulated by *Chuk* or *Ikbkb* gene silencing (A). The central region labeled P > 0.05 in (A) contains 131 genes and was not interpreted as a significant response category. PCA biplot showing the relative gene contribution grouped by *Chuk* or *Ikbkb* silencing conditions and revealing the specificity of gene-silencing target (*Chuk*, *Ikbkb* or control) (B). Heatmap describing the unsupervised hierarchical clustering of the 188 differentially expressed genes by cell line and gene-silencing target (C).

4. Discussion

This study is the first to investigate the involvement of IKK α and IKK β kinases associated with NF- κ B signaling in tumor formation using in vitro and in vivo MPM murine models. Based on the cytoplasmic vs. nuclear presence of the NF- κ B-related signaling subunits (RelB and p52), there seems to be a predominance of the non-canonical activation pathway for both murine and human MPM cell models, but this finding needs to be further confirmed. We first used three mouse models of MPM, two chemically induced (AB2, AE17) and one genetically induced (KPM1), and showed that both IKK α and IKK β were expressed. Their participation in disease progression was then explored by silencing *Chuk* and *Ikbkb*, the genes that encode IKK α and IKK β , respectively. We found that both kinases were important for in vitro malignant cell proliferation. IKK α seemed to be highly

associated with tumor cell survival in the presence of *Kras* mutations (AE17 and KPM1 cell models), suggesting a preferential involvement of the *Chuk* signaling pathway in the presence of this mutation. In vivo, both kinases were important for primary tumor formation. IKK α was significantly implicated in all MPM mouse models, whereas IKK β impact in disease was more variable across models. Translating our results from a human cohort, we found that both canonical and atypical NF- κ B signaling pathways that include *CHUK* and *IKBKB*, characterize a specific cluster of malignant samples, highlighting the importance of these genes as part of the mechanisms underlying MPM.

There is little evidence about the mechanistic implication of NF- κ B in mesothelioma. One other study showed a possible protective role of both kinases in the survival and transformation of human mesothelial cells in malignant mesothelioma elicited by asbestos exposure-related TNF- α release [29]. Our findings support these previous observations and, because of we used of non-isogenic cell lines, raise the possibility that the contribution of IKK α to MPM cell survival may vary depending on the genetic background of the tumor cells. This is because AE17 and KPM1 cells carry different *Kras* alterations, with KPM1 cells also having a *Trp53* deletion and AB2 cells also being different in terms of strain background and tumor origin. Similarly, our exploratory transcriptomic analyses support the involvement of both canonical and atypical (non-canonical) NF- κ B-associated gene signaling in MPM tumors, unfortunately without establishing specific diagnostic performance or tumor-cell-intrinsic *CHUK/IKBKB* activity. In-depth genomic, transcriptomic, multi-omic, and pharmacogenomic approaches may help refine future MPM classification and treatment prioritization, and prospective clinical validation remains necessary [11–13].

The activation of NF- κ B signaling in exposure to asbestos and induced chronic inflammation is also associated with malignant transformations and resistance to chemotherapy [1,29,49]. So far, studies have focused on the inhibition of cell proliferation or the induction of apoptosis by targeting NF- κ B-associated signaling pathways, such as in vivo SOCS1 gene delivery combined with cisplatin plus pemetrexed or in vitro ALDH1A3 expression modulation in the targeting of STAT3-NF- κ B activity [49,50]. Moreover, the in vitro treatment of MPM cell lines with ranpirnase/Onconase, a cytotoxic antitumor ribonuclease, was reported to downregulate NF- κ B/NFKB1-related signaling and decrease cell proliferation and invasion in MPM cell lines [51]. However, direct evidence regarding NF- κ B in promoting mesothelioma formation has been insufficient. Only a few reports revealed NF- κ B activation in mesothelioma cells in the presence of proteasome inhibitors or antitumor ribonuclease reagents (including IKK β inhibitor IMD-0354) that may prevent NF- κ B activity and lead to the effective inhibition of tumor proliferation and the induction of apoptosis [52–55]. However, these reagents may also influence the degradation and synthesis of a wide range of functional proteins. Therefore, the development of effective drugs with fewer side effects is necessary.

Although previous reports suggested an important role of NF- κ B activation in mesothelioma [29,49,51,52], the evidence is sparse. Here, we show that both IKK α and IKK β may be important in different aspects of cell proliferation and disease progression in vivo and possibly important in human malignancy as well. *Chuk*-signaling, more than *Ikbkb*-signaling, seemed associated with phenotypic changes in apoptosis, MTT reduction, clonogenicity, and several in vivo tumor development parameters. These findings suggest prioritizing further mechanistic investigation of the *Chuk*-linked phenotypes and the better characterization of the role of IKK α as a potential therapeutic target in MPM.

There are several limitations to this study. First, the gene-silencing experiments relied on single shRNA reagents that produced cross-target mRNA effects, without independent shRNAs or rescue experiments, thereby limiting target-specific attribution. Second, sh*Chuk*-associated apoptosis was observed in the two *Kras*-mutant cell lines but not in AB2,

suggesting a potential relationship with *Kras* mutation and *Trp53* status that requires further validation in isogenic models. Third, knockdown was assessed primarily by qPCR and qualitative immunofluorescence, without quantitative immunoblotting or comprehensive functional analysis of NF- κ B signaling, including IKK phosphorylation, I κ B α degradation, p100 processing, NF- κ B nuclear translocation, and transcriptional activity. These limitations prevent precise assignment of the tumor phenotypes as being dependent on IKK α or IKK β signaling. Future studies should therefore incorporate independent shRNAs, genetic rescue, endpoint confirmation of target suppression, orthogonal readouts of canonical and non-canonical NF- κ B signaling, validation of candidate transcriptional responses, and more appropriate human pleural controls.

5. Conclusions

In conclusion, the present study supports a role for IKK-associated signaling in the maintenance and progression of experimental MPM. Both IKK α and IKK β contributed to cellular fitness and tumor development in selected settings, but IKK α depletion generated the most consistent effects on apoptosis, clonogenic survival and in vivo tumor growth. The findings support IKK α as a therapeutic candidate and raise the possibility of its interaction with oncogenic *KRAS* signaling.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cancers18182990/s1>, Table S1: Lentiviral shRNA pools used for these studies; Table S2: Antibodies used for these studies; Table S3: PCR primers used for these studies; Table S4: Raw mouse group sizes for MPM mouse experiments; Figure S01: Figure 1C—RelA, cytosol (65 kDa); Figure S02: Figure 1C—RelA, nucleus (65 kDa); Figure S03: Figure 1C—RelB, cytosol and nucleus (68 kDa); Figure S04: Figure 1C—p52, cytosol (52 kDa); Figure S05: Figure 1C—p52, nucleus (52 kDa); Figure S06: Figure 1C— β -actin, cytosol (43 kDa); Figure S07: Figure 1C— β -actin, nucleus (43 kDa). Figure S08: Uncropped image of Figure 1B.

Author Contributions: Conceptualization, [I.G., T.A.A., I.E.F. and G.T.S.]; Methodology, [B.D., I.G., I.L., J.J., M.L., D.S., G.N., T.A.A., I.E.F. and G.T.S.]; Validation, [B.D., I.G., I.L., J.J., M.L., D.S., G.N., T.A.A., I.E.F. and G.T.S.]; Formal Analysis, [I.G., I.L., I.E.F. and G.T.S.]; Investigation, [B.D., I.G., I.L., J.J., M.L., D.S., G.N., T.A.A., I.E.F. and G.T.S.]; Data Curation, [B.D., I.G., I.L., J.J., M.L., D.S., G.N., T.A.A., I.E.F. and G.T.S.]; Writing—Original Draft Preparation, Review and Editing, [B.D., I.G., L.A., I.L., J.J., M.L., D.S., G.N., T.A.A., I.E.F. and G.T.S.]; Visualization, [B.D., I.G., L.A., I.L., J.J., I.E.F. and G.T.S.]; Supervision, [I.E.F. and G.T.S.]; Project Administration, [T.A.A., I.E.F. and G.T.S.]; Funding Acquisition, [I.E.F. and G.T.S.]. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Prefecture of Western Greece's Veterinary Administration (protocol code 118018/578) and was conducted in accordance with Directive 2010/63/EU, approved on 30 April 2014.

Informed Consent Statement: Not applicable. This study did not enroll human participants and used publicly available de-identified human expression data.

Data Availability Statement: The publicly available human expression dataset analyzed in this study is available in the Gene Expression Omnibus under accession GSE51024. The RNA-sequencing data reported for the murine cell-line experiments are deposited under accession GSE129984.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Carbone, M.; Yang, H. Molecular Pathways: Targeting Mechanisms of Asbestos and Erionite Carcinogenesis in Mesothelioma. *Clin. Cancer Res.* **2012**, *18*, 598–604. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Carbone, M.; Ly, B.H.; Dodson, R.F.; Pagano, I.; Morris, P.T.; Dogan, U.A.; Gazdar, A.F.; Pass, H.I.; Yang, H. Malignant Mesothelioma: Facts, Myths, and Hypotheses. *J. Cell. Physiol.* **2012**, *227*, 44–58. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Fu, L.; Liu, K.; Liu, Y.; Gao, Y.; Wang, B.; Sun, Y.; Shu, W.; Ning, Y.; Zhang, M.; Du, J.; et al. Global Patterns and Trends in Mesothelioma Incidence: A Retrospective Cross-Sectional Study. *Lung Cancer* **2026**, *211*, 108878. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Bray, F.; Laversanne, M.; Sung, H.; Ferlay, J.; Siegel, R.L.; Soerjomataram, I.; Jemal, A. Global Cancer Statistics 2022: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2024**, *74*, 229–263. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Kindler, H.L.; Ismaila, N.; Bazhenova, L.; Chu, Q.; Churpek, J.E.; Dagogo-Jack, I.; Bryan, D.S.; Drazer, M.W.; Forde, P.; Husain, A.N.; et al. Treatment of Pleural Mesothelioma: ASCO Guideline Update. *J. Clin. Oncol.* **2025**, *43*, 1006–1038. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Baas, P.; Scherpereel, A.; Nowak, A.K.; Fujimoto, N.; Peters, S.; Tsao, A.S.; Mansfield, A.S.; Popat, S.; Jahan, T.; Antonia, S.; et al. First-Line Nivolumab plus Ipilimumab in Unresectable Malignant Pleural Mesothelioma (CheckMate 743): A Multicentre, Randomised, Open-Label, Phase 3 Trial. *Lancet* **2021**, *397*, 375–386. Erratum in *Lancet* **2021**, *20*, 670. [https://doi.org/10.1016/S0140-6736\(21\)00369-X](https://doi.org/10.1016/S0140-6736(21)00369-X). [\[CrossRef\]](#) [\[PubMed\]](#)
7. Scherpereel, A.; Baas, P.; Nowak, A.K.; Tsao, A.S.; Fujimoto, N.; Peters, S.; Mansfield, A.S.; Popat, S.; Bautista Aragon, Y.; Talbot, T.; et al. Five-Year Clinical Outcomes with Nivolumab Plus Ipilimumab Versus Chemotherapy as First-Line Treatment for Unresectable Pleural Mesothelioma in CheckMate 743. *J. Clin. Oncol.* **2026**, *44*, 742–749. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Treasure, T. What Is the Best Approach for Surgery of Malignant Pleural Mesothelioma? It Is to Put Our Efforts into Obtaining Trustworthy Evidence for Practice. *J. Thorac. Cardiovasc. Surg.* **2016**, *151*, 307–309. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Dhalluin, X.; Scherpereel, A. Treatment of Malignant Pleural Mesothelioma: Current Status and Future Directions. *Monaldi Arch. Chest Dis.* **2010**, *73*, 79–85. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Scherpereel, A.; Astoul, P.; Baas, P.; Berghmans, T.; Clayson, H.; de Vuyst, P.; Dienemann, H.; Galateau-Salle, F.; Hennequin, C.; Hillerdal, G.; et al. Guidelines of the European Respiratory Society and the European Society of Thoracic Surgeons for the management of malignant pleural mesothelioma. *Zhongguo Fei Ai Za Zhi* **2010**, *13*, C23–C45. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Bueno, R.; Stawiski, E.W.; Goldstein, L.D.; Durinck, S.; De Rienzo, A.; Modrusan, Z.; Gnad, F.; Nguyen, T.T.; Jaiswal, B.S.; Chirieac, L.R.; et al. Comprehensive Genomic Analysis of Malignant Pleural Mesothelioma Identifies Recurrent Mutations, Gene Fusions and Splicing Alterations. *Nat. Genet.* **2016**, *48*, 407–416. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Hmelfjak, J.; Sanchez-Vega, F.; Hoadley, K.A.; Shih, J.; Stewart, C.; Heiman, D.; Tarpey, P.; Danilova, L.; Drill, E.; Gibb, E.A.; et al. Integrative Molecular Characterization of Malignant Pleural Mesothelioma. *Cancer Discov.* **2018**, *8*, 1548–1565. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Assié, J.-B.; Meiller, C.; Stern, E.; Lasvergnas, J.; Arnould, M.; Pan, L.; Montagne, F.; Sequeiros, R.; Al Zreibi, C.; Del Nery, E.; et al. Pharmacogenomic Characterization of a Large Cohort of Patient-Derived Cell Lines Identifies Therapeutic Strategies for Pleural Mesothelioma. *Cancer Res.* **2026**, *86*, 196–212. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Mangiante, L.; Alcalá, N.; Sexton-Oates, A.; Di Genova, A.; Gonzalez-Perez, A.; Khandekar, A.; Bergstrom, E.N.; Kim, J.; Liu, X.; Blazquez-Encinas, R.; et al. Multiomic Analysis of Malignant Pleural Mesothelioma Identifies Molecular Axes and Specialized Tumor Profiles Driving Intertumor Heterogeneity. *Nat. Genet.* **2023**, *55*, 607–618. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Quispel-Janssen, J.M.; Badhai, J.; Schunselaar, L.; Price, S.; Brammell, J.; Iorio, F.; Kolluri, K.; Garnett, M.; Berns, A.; Baas, P.; et al. Comprehensive Pharmacogenomic Profiling of Malignant Pleural Mesothelioma Identifies a Subgroup Sensitive to FGFR Inhibition. *Clin. Cancer Res.* **2018**, *24*, 84–94. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Lin, Y.; Bai, L.; Chen, W.; Xu, S. The NF-kappaB Activation Pathways, Emerging Molecular Targets for Cancer Prevention and Therapy. *Expert Opin. Ther. Targets* **2010**, *14*, 45–55. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Giopanou, I.; Lilis, I.; Papaleonidopoulos, V.; Marazioti, A.; Spella, M.; Vreka, M.; Papadaki, H.; Stathopoulos, G.T. Comprehensive Evaluation of Nuclear Factor-kappaBeta Expression Patterns in Non-Small Cell Lung Cancer. *PLoS ONE* **2015**, *10*, e0132527. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Zhou, S.; Ye, W.; Shao, Q.; Qi, Y.; Zhang, M.; Liang, J. Prognostic Significance of XIAP and NF-kappaB Expression in Esophageal Carcinoma with Postoperative Radiotherapy. *World J. Surg. Oncol.* **2013**, *11*, 288. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Smith, S.M.; Lyu, Y.L.; Cai, L. NF-kappaB Affects Proliferation and Invasiveness of Breast Cancer Cells by Regulating CD44 Expression. *PLoS ONE* **2014**, *9*, e106966. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Hoffmann, A.; Cheng, G.; Baltimore, D. NF-κB: Master Regulator of Cellular Responses in Health and Disease. *Immun. Inflamm.* **2025**, *1*, 2. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Chen, F.; Castranova, V.; Shi, X.; Demers, L.M. New Insights into the Role of Nuclear Factor-kappaB, a Ubiquitous Transcription Factor in the Initiation of Diseases. *Clin. Chem.* **1999**, *45*, 7–17. [\[CrossRef\]](#)

22. Hoffmann, A.; Natoli, G.; Ghosh, G. Transcriptional Regulation via the NF-kappaB Signaling Module. *Oncogene* **2006**, *25*, 6706–6716. [[CrossRef](#)] [[PubMed](#)]
23. Besta, S.; Samuels, M.; Roupakia, E.; Kanellis, D.C.; Marcu, K.B.; Kolettas, E. The Roles of IKK α in Normal Physiology and Cancer. *Cell Commun. Signal* **2026**, *24*, 330. [[CrossRef](#)] [[PubMed](#)]
24. Tegowski, M.; Baldwin, A. Noncanonical NF- κ B in Cancer. *Biomedicines* **2018**, *6*, 66. [[CrossRef](#)] [[PubMed](#)]
25. Zhang, J.; Zhang, R.; Li, W.; Ma, X.-C.; Qiu, F.; Sun, C.-P. I κ B Kinase β (IKK β): Structure, Transduction Mechanism, Biological Function, and Discovery of Its Inhibitors. *Int. J. Biol. Sci.* **2023**, *19*, 4181–4203. [[CrossRef](#)] [[PubMed](#)]
26. Li, J.; Peet, G.W.; Pullen, S.S.; Schembri-King, J.; Warren, T.C.; Marcu, K.B.; Kehry, M.R.; Barton, R.; Jakes, S. Recombinant I κ B Kinases α and β Are Direct Kinases of I κ B α . *J. Biol. Chem.* **1998**, *273*, 30736–30741. [[CrossRef](#)] [[PubMed](#)]
27. Liu, S.; Chen, Z.; Zhu, F.; Hu, Y. I κ B Kinase Alpha and Cancer. *J. Interferon Cytokine Res.* **2012**, *32*, 152–158. [[CrossRef](#)] [[PubMed](#)]
28. Tinto, K.; Cunningham, M.; Plevin, R. Double Trouble: Cytosolic and Nuclear IKK α in Cancer. *Open Biol.* **2025**, *15*, 240375. [[CrossRef](#)] [[PubMed](#)]
29. Yang, H.; Bocchetta, M.; Kroczyńska, B.; Elmishad, A.G.; Chen, Y.; Liu, Z.; Bubici, C.; Mossman, B.T.; Pass, H.I.; Testa, J.R.; et al. TNF-Alpha Inhibits Asbestos-Induced Cytotoxicity via a NF-kappaB-Dependent Pathway, a Possible Mechanism for Asbestos-Induced Oncogenesis. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10397–10402. [[CrossRef](#)] [[PubMed](#)]
30. Comar, M.; Zanotta, N.; Bonotti, A.; Tognon, M.; Negro, C.; Cristaudo, A.; Bovenzi, M. Increased Levels of C-C Chemokine RANTES in Asbestos Exposed Workers and in Malignant Mesothelioma Patients from an Hyperendemic Area. *PLoS ONE* **2014**, *9*, e104848. [[CrossRef](#)] [[PubMed](#)]
31. Robinson, B.W.S.; Creaney, J.; Lake, R.; Nowak, A.; Musk, A.W.; de Klerk, N.; Winzell, P.; Hellstrom, K.E.; Hellstrom, I. Mesothelin-Family Proteins and Diagnosis of Mesothelioma. *Lancet* **2003**, *362*, 1612–1616. [[CrossRef](#)] [[PubMed](#)]
32. Jackson, E.L.; Willis, N.; Mercer, K.; Bronson, R.T.; Crowley, D.; Montoya, R.; Jacks, T.; Tuveson, D.A. Analysis of Lung Tumor Initiation and Progression Using Conditional Expression of Oncogenic K-Ras. *Genes Dev.* **2001**, *15*, 3243–3248. [[CrossRef](#)] [[PubMed](#)]
33. Marino, S.; Vooijs, M.; van Der Gulden, H.; Jonkers, J.; Berns, A. Induction of Medulloblastomas in P53-Null Mutant Mice by Somatic Inactivation of Rb in the External Granular Layer Cells of the Cerebellum. *Genes Dev.* **2000**, *14*, 994–1004. [[CrossRef](#)]
34. Jackaman, C.; Bundell, C.S.; Kinnear, B.F.; Smith, A.M.; Fillion, P.; van Hagen, D.; Robinson, B.W.S.; Nelson, D.J. IL-2 Intratumoral Immunotherapy Enhances CD8⁺ T Cells That Mediate Destruction of Tumor Cells and Tumor-Associated Vasculature: A Novel Mechanism for IL-2. *J. Immunol.* **2003**, *171*, 5051–5063. [[CrossRef](#)] [[PubMed](#)]
35. Marazioti, A.; Krontira, A.C.; Behrend, S.J.; Giotopoulou, G.A.; Ntaliarda, G.; Blanquart, C.; Bayram, H.; Iliopoulou, M.; Vreka, M.; Trassl, L.; et al. KRAS Signaling in Malignant Pleural Mesothelioma. *EMBO Mol. Med.* **2022**, *14*, e13631. [[CrossRef](#)] [[PubMed](#)]
36. Giopanou, I.; Lilis, I.; Papaleonidopoulos, V.; Agalioti, T.; Kanellakis, N.I.; Spiropoulou, N.; Spella, M.; Stathopoulos, G.T. Tumor-Derived Osteopontin Isoforms Cooperate with TRP53 and CCL2 to Promote Lung Metastasis. *Oncoimmunology* **2017**, *6*, e1256528. [[CrossRef](#)] [[PubMed](#)]
37. Giannou, A.D.; Marazioti, A.; Kanellakis, N.I.; Giopanou, I.; Lilis, I.; Zazara, D.E.; Ntaliarda, G.; Kati, D.; Armenis, V.; Giotopoulou, G.A.; et al. NRAS Destines Tumor Cells to the Lungs. *EMBO Mol. Med.* **2017**, *9*, 672–686. [[CrossRef](#)] [[PubMed](#)]
38. Spella, M.; Lilis, I.; Pepe, M.A.; Chen, Y.; Armaka, M.; Lamort, A.-S.; Zazara, D.E.; Roumelioti, F.; Vreka, M.; Kanellakis, N.I.; et al. Club Cells Form Lung Adenocarcinomas and Maintain the Alveoli of Adult Mice. *eLife* **2019**, *8*, e45571. [[CrossRef](#)] [[PubMed](#)]
39. Marazioti, A.; Lilis, I.; Vreka, M.; Apostolopoulou, H.; Kalogeropoulou, A.; Giopanou, I.; Giotopoulou, G.A.; Krontira, A.C.; Iliopoulou, M.; Kanellakis, N.I.; et al. Myeloid-Derived Interleukin-1 β Drives Oncogenic KRAS-NF- κ B Addiction in Malignant Pleural Effusion. *Nat. Commun.* **2018**, *9*, 672. [[CrossRef](#)] [[PubMed](#)]
40. Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2^{- $\Delta\Delta$ CT} Method. *Methods* **2001**, *25*, 402–408. [[CrossRef](#)] [[PubMed](#)]
41. Ritchie, M.E.; Phipson, B.; Wu, D.; Hu, Y.; Law, C.W.; Shi, W.; Smyth, G.K. Limma Powers Differential Expression Analyses for RNA-Sequencing and Microarray Studies. *Nucleic Acids Res.* **2015**, *43*, e47. [[CrossRef](#)] [[PubMed](#)]
42. McCarthy, D.J.; Chen, Y.; Smyth, G.K. Differential Expression Analysis of Multifactor RNA-Seq Experiments with Respect to Biological Variation. *Nucleic Acids Res.* **2012**, *40*, 4288–4297. [[CrossRef](#)] [[PubMed](#)]
43. Edgar, R.; Domrachev, M.; Lash, A.E. Gene Expression Omnibus: NCBI Gene Expression and Hybridization Array Data Repository. *Nucleic Acids Res.* **2002**, *30*, 207–210. [[CrossRef](#)] [[PubMed](#)]
44. Fridlender, Z.G.; Sun, J.; Kim, S.; Kapoor, V.; Cheng, G.; Ling, L.; Worthen, G.S.; Albelda, S.M. Polarization of Tumor-Associated Neutrophil Phenotype by TGF-Beta: “N1” versus “N2” TAN. *Cancer Cell* **2009**, *16*, 183–194. [[CrossRef](#)] [[PubMed](#)]
45. Agalioti, T.; Giannou, A.D.; Krontira, A.C.; Kanellakis, N.I.; Kati, D.; Vreka, M.; Pepe, M.; Spella, M.; Lilis, I.; Zazara, D.E.; et al. Mutant KRAS Promotes Malignant Pleural Effusion Formation. *Nat. Commun.* **2017**, *8*, 15205. [[CrossRef](#)] [[PubMed](#)]
46. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An Open-Source Platform for Biological-Image Analysis. *Nat. Methods* **2012**, *9*, 676–682. [[CrossRef](#)]

47. Suraokar, M.B.; Nunez, M.I.; Diao, L.; Chow, C.W.; Kim, D.; Behrens, C.; Lin, H.; Lee, S.; Raso, G.; Moran, C.; et al. Expression Profiling Stratifies Mesothelioma Tumors and Signifies Deregulation of Spindle Checkpoint Pathway and Microtubule Network with Therapeutic Implications. *Ann. Oncol.* **2014**, *25*, 1184–1192. [[CrossRef](#)] [[PubMed](#)]
48. Schaefer, C.F.; Anthony, K.; Krupa, S.; Buchoff, J.; Day, M.; Hannay, T.; Buetow, K.H. PID: The Pathway Interaction Database. *Nucleic Acids Res.* **2009**, *37*, D674–D679. [[CrossRef](#)] [[PubMed](#)]
49. Canino, C.; Luo, Y.; Marcato, P.; Blandino, G.; Pass, H.I.; Cioce, M. A STAT3-NFkB/DDIT3/CEBP β Axis Modulates ALDH1A3 Expression in Chemoresistant Cell Subpopulations. *Oncotarget* **2015**, *6*, 12637–12653. [[CrossRef](#)] [[PubMed](#)]
50. Iwahori, K.; Serada, S.; Fujimoto, M.; Ripley, B.; Nomura, S.; Mizuguchi, H.; Shimada, K.; Takahashi, T.; Kawase, I.; Kishimoto, T.; et al. SOCS-1 Gene Delivery Cooperates with Cisplatin plus Pemetrexed to Exhibit Preclinical Antitumor Activity against Malignant Pleural Mesothelioma. *Int. J. Cancer* **2013**, *132*, 459–471. [[CrossRef](#)] [[PubMed](#)]
51. Goparaju, C.M.; Blasberg, J.D.; Volinia, S.; Palatini, J.; Ivanov, S.; Donington, J.S.; Croce, C.; Carbone, M.; Yang, H.; Pass, H.I. Onconase Mediated NFK β Downregulation in Malignant Pleural Mesothelioma. *Oncogene* **2011**, *30*, 2767–2777. [[CrossRef](#)] [[PubMed](#)]
52. Tanaka, A.; Konno, M.; Muto, S.; Kambe, N.; Morii, E.; Nakahata, T.; Itai, A.; Matsuda, H. A Novel NF-kappaB Inhibitor, IMD-0354, Suppresses Neoplastic Proliferation of Human Mast Cells with Constitutively Activated c-Kit Receptors. *Blood* **2005**, *105*, 2324–2331. [[CrossRef](#)] [[PubMed](#)]
53. Matsuda, A.; Tanaka, A.; Muto, S.; Ohmori, K.; Furusaka, T.; Jung, K.; Karasawa, K.; Okamoto, N.; Oida, K.; Itai, A.; et al. A Novel NF- κ B Inhibitor Improves Glucocorticoid Sensitivity of Canine Neoplastic Lymphoid Cells by up-Regulating Expression of Glucocorticoid Receptors. *Res. Vet. Sci.* **2010**, *89*, 378–382. [[CrossRef](#)] [[PubMed](#)]
54. Cerruti, F.; Jocolle, G.; Salio, C.; Oliva, L.; Paglietti, L.; Alessandria, B.; Mioletti, S.; Donati, G.; Numico, G.; Cenci, S.; et al. Proteasome Stress Sensitizes Malignant Pleural Mesothelioma Cells to Bortezomib-Induced Apoptosis. *Sci. Rep.* **2017**, *7*, 17626. [[CrossRef](#)] [[PubMed](#)]
55. Nishikawa, S.; Tanaka, A.; Matsuda, A.; Oida, K.; Jang, H.; Jung, K.; Amagai, Y.; Ahn, G.; Okamoto, N.; Ishizaka, S.; et al. A Molecular Targeting against Nuclear Factor- κ B, as a Chemotherapeutic Approach for Human Malignant Mesothelioma. *Cancer Med.* **2014**, *3*, 416–425. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.