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ARAŞTIRMA / RESEARCH

NF-Kappa B expression in pancreatic ductal carcinoma

Pankreas duktal adenokarsinomunda NF-Kappa B ekspresyonu

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Abstract

Purpose: In this study we investigated the expression of the p65 subunit of the nuclear factor-kappaB (NF-kB) complex and the activation status of NF-ĸB by phospho-IĸB-alpha antibody.

Materials and Methods: A tissue microarray based on material obtained from 107 patients was utilized. The antibody staining was scored by combining staining intensity with percentage of tumor staining. The antibodies used were NF-kB p65 and phospho-IkB-alpha(ser32/36), both from Cell Signaling Technology. The staining scores were correlated with the archival data available on some patients on margin and lymph node status, stage, tumor size, as well as clinical data including survival.

Results: The staining was nuclear (p65) and cytoplasmic (p-IĸBalpha) respectively. In general there was an increased expression and activation of NF-kB in the carcinomas, compared to non-tumoral regions. None of the markers had a significant correlation with the overall survival. NF-ĸB(p65) expression had a correlation with positive lymph node status.

Conclusion: The correlation with the positive lymph node status suggests a role in invasive properties of the tumor. Activation of NF-kB is most likely an early event in pancreatic carcinogenesis. Despite the lack of an effect on overall survival, due to its increased activation in pancreatic cancer, NF-ĸB is still a good target for therapeutic interventions.

Keywords: NF-Kappa B, pancreas, adenocarcinoma

Öz

Amaç: Bu çalışmada, NF-KB kompleksinin alt grubu olan p65 ve fosfo-IKB-alfa antikor ile NF-KB'nin aktivasyonu durumu araştırılmıştır.

Gereç ve Yöntem: Doku mikroarray yöntemi ile 107 olgu üzerinden yapılan çalışmada antikorun boyanması, boyanma şiddeti ve yüzdesi beraber değerlendirilerek skorlandı. Cell Signaling Technology ürünlerinden p65 ve fosfo-IKB-alfa(ser32/36) kullanıldı. Boyanma skorlarının arşiv bilgileri doğrultusunda cerrahi sınır, lenf bezi durumu, evre, tümör boyutu ve klinik bilgiler ışığında genel yaşam süresi ile korelasyonu araştırıldı.

Bulgular: P65 nükleer, p-IĸB-alfa sitoplazmik boyanma paterni izledi. Karsinomda, tümör içermeyen bölgelerle kıyaslandığında boyanma belirgin oranda şiddetli olup ve NF-KB aktivitesinde artış mevcuttu. NF-ĸB(p65) ekspresyonu, lenf bezi durumu ile anlamlı bir korelasyon göstermekle birlikte yaş, ırk, cinsiyet, tümör lokalizasyonu, tümör boyutu, tümör derecesi ve genel yaşam süresi ile NF-KB(p65), pIkBalfa arasında korelasyon izlenmedi.

Sonuç: Lenf bezi durumu ile NF-KB aktivitesinin korelasyonu tümörün invaziv olma özelliğini ön plana çıkartmakta olup pankreas karsinogenezinde erken aşamalarda görülmesi tedavi açılımlarının geliştirilmesi açısından hedef molekül olabileceğini düşündürmektedir.

Anahtar kelimeler: NF-Kappa B, pankreas, adenokarsinom.

INTRODUCTION

Pancreatic adenocarcinoma (PAC) is a deadly disease characterized by late diagnosis, early metastasis, and resistance to therapy. It is one of the most aggressive tumor with has parallelity between mortality and incidence rate[1]. Recent advances in surgical and medical therapy have had little impact on the mortality rate of this disease[2].

K-ras gene is the most frequent altered oncogene in

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pancreatic cancer[3,4] . This mutation is present in up to 90 % of pancreatic cancer cases[5]. The expression of mutant K-ras activates the Akt/protein kinase B (IKKs) pathway, resulting in the activation of the nuclear factor-kappaB (NF-kappaB) transcriptional factor which is the generic name of a family of transcription factors that act as dimers and regulate genes involved in cell growth, survival and differentiation[6,7] . The NF-kB family comprises five subunits[8]: p65, c-Rel, Rel-B, NF-kB1 and NF-kB2[9-14]. In most cells, the major proportion of NF-kB proteins resides in the cytoplasm in a latent state[15].

The activation of NF-kB can be divided into two phases. The first phase takes place in the cytoplasm and leads to the activation of a kinase complex composed of three subunits: IkB kinase(IKK)a, IKKb and NEMO/IKKg. This IKK complex phosphorylates IkB proteins leading to their ubiquitination and subsequent proteasome-dependent degradation, the main consequence of which is the translocation of NF-kB dimers to the nucleus[9,11]. The second phase occurs in the nucleus and involves post translocation modifications of NF-kB subunits, which are required to regulate the transcriptional activity.

The constitutive activation of the transcription factor nuclear-factor kappa B (NF-kappaB) in the nucleus of the cells regulates its downstream genes to promote cancer cell growth due to the activation of gene transcription, including genes encoding inflammatory cytokines, chemokines, growth factors, cell adhesion molecules, and cytokine reseptors[16,17]. Therefore, it is a hallmark of many highly malignant tumors including breast[18-21], ovarian[21,22], colon[21], bladder[18], prostate carcinoma[23-25], melanoma[26], as well as pancreatic ductal adenocarcinoma[7,27,28].

It is also possible that constitutive NF-KB activity is required for tumorigenesis at the early stage of pancreatic cancer development. It plays an essential role in the initiation or progression of malignant transformation of pancreatic ductal epithelial cells by promoting tumor cell survival towards malignancy[29].

NF-kappaB has also been shown to inhibit apoptosis in response to chemotherapeutic agents[30-36]. In order to understand the clinical significance of NF-KB in pancreatic cancer, we examined the expression of NF-KB in pancreatic ductal adenocarcinoma by immunochemistry, and analyzed the correlation of NF-KB expression with margin, lymph node status, stage, tumor size, and survival.

MATERIALS AND METHODS

A total of 107 ordinary PDA (pancreas ductal adenocarcinoma) cases, for which the tissue was available for immunohistochemical staining, were identified. Other tumor types, non-ductal neoplasia, CBD, and ampullary tumors were carefully excluded. Routine formalin-fixed, paraffin-embedded and H&E stained sections were reviewed. Tissue microarrays were prepared by obtaining 3 samples of each 1.4 mm-diameter from each tumor. Demographic data including age, gender, race, and tumor location was obtained from the surgical pathology reports, patients' charts, and clinical databases.

Histological grading

The ductal adenocarcinomas were graded according to the recently proposed scheme (37). Briefly, well-formed tubular units with complete, easily discernible borders were regarded as grade 1. Those with incomplete, ill-defined borders, fusion of glands or irregular multi-lumina formation (cribriform architecture) were categorized as grade 2. Non-glandular patterns including cord-like areas, individual cell infiltration, nested or solid (sheet-like) growth patterns were classified as grade 3.

NF-KB immunostaining

Immunohistochemistry was performed on 5 μm tissue sections. Sections were deparaffinized and hydrated through d.H2O. Endogenous peroxidase blocked with a 3% Hydrogen peroxide (20 min.). Followed by antigen retrieval with citrate buffer Ph 7.0 in steamer, slides were immersed in hot buffer (20 min) and then remained in warm buffer (20 min.). Blocking was performed in 5% normal rabbit serum (30 min). Incubation of primary antibody NF-KB p65 (1:100 dilution, Cell Signaling, NF-κB p65 (C22B4) Rabbit mAb #4764) and Phospho-IkappaB-alpha (Ser32/36) (1:100 dilution, Cell Signaling, Phospho-IkappaB-alpha (Ser32/36) (5A5) Monoclonal Ab #5205) was done at room temperature(2 hours) was followed by incubation in the Anti-goat biotinylated secondary antibody, IgG (H+L) (Vector Lab, 30 minutes), and incubation in avidin-biotin complex (Vector Lab, 30 minutes) according to manufacture's manual instruction. Visualization was performed with a DAB substrate/chromagen and counterstained with hematoxylin.

Immunohistochemical grading

An established scoring system that evaluates both the percent and intensity was utilized. Briefly, the percent of cells staining is graded as: 0=less than 1 % of the cells positive, 1=1 to 10 % of the cells positive, 2=11 to 50 % of the cells positive, 3=51 to 80 % of the cells positive and 4=more than 80 % of the cells positive. The intensity defined as: 1=weak, 2=moderate, 3=strong. The overall score is then calculated as (1+intensity/3) x percent, and for comparative analysis the data was arbitrarily divided into four groups: 0=none, 1=minimal, 2=moderate, and 3=significant.

Table 1. The correlation of NF-k B(p65) with lymph node status, grade and tumor size

NF-k B(p65) vs.	Spearman C	p
Phospho-IkBa	0.050	0.09
Lymph node status	0.220	0.04
Grade	0.112	0.30
Tumor size (>5.0 cm)	0.032	0.77

Statistical analysis

The expression of NF-KB was compared with clinico-pathological features and known prognostic parameters of ductal adenocarcinoma including tumor size, grade, resection margins and lymph node status. All statistical tests were performed using the SPS 10.0 data analysis program. Data were analyzed using the chi-Spearman C test to compare the expression of NF-KB with tumor size, grade, resection margins and lymph node status. Overall survival data were analyzed by using the Kaplan-Meier method and were assessed by log-rank test to compare differences in survival between the patients whose tumors expressed strong positive NF-KB staining and those whose tumors were either negative or stained weakly for NF-KB. By using the proportional hazard model of Cox, multivariate analysis was performed on NF-KB expression, tumor size, grade, margin status and lymph node metastasis in patients with ductal adenocarcinoma. $P < 0.05$ was regarded as statistically significant.

RESULTS

The mean age of the 107 patients with PAC, at time of diagnosis was 64 years (range 36-86). 66 of these patients were female and 41 were male. 90 patients had tumor in the head region while 17 had tumor in the tail. Average size of the tumors was 3.5 cms (range= 0.8-9 cm). 84 patients had lesions that measured more than 2 cms. 25/103 had lymph node metastases. Median survival was 11 months. At this time of this presentation, 18 cases were dead and 89 cases are still alive.

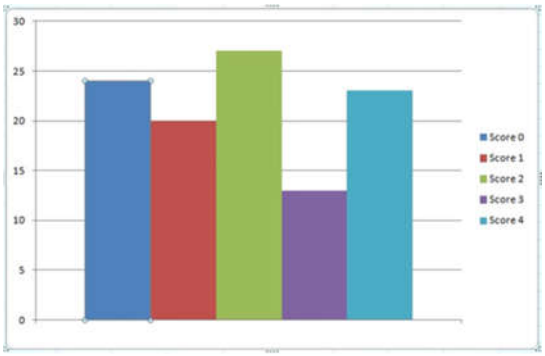
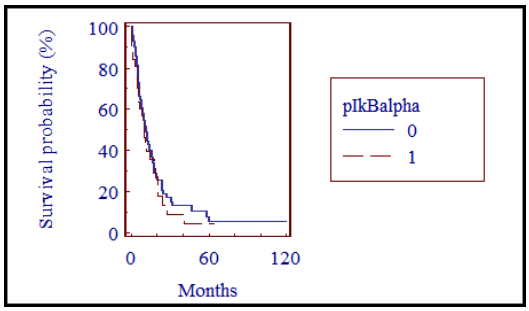


Figure 1.The NF-kB(p65) expression scores in the carcinoma samples



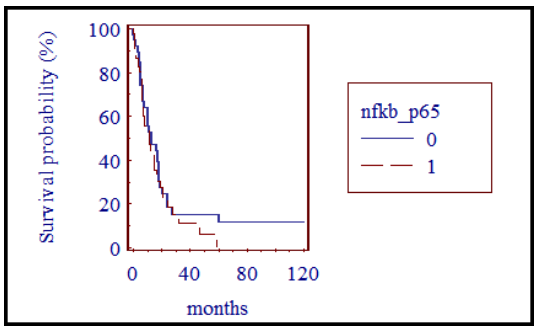


Figure 2. The correlation of pIkBalpha and NF-kB(p65) with the overall survival

NF-KB expression

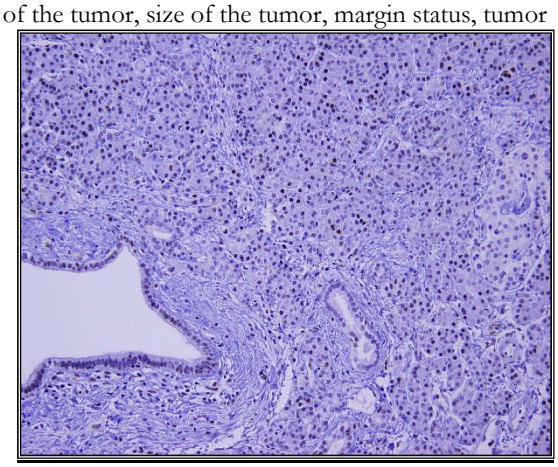
In normal tissue: In normal pancreas, NF-KB was expressed weakly in the ducts, and focally in the acini (Picture 1,2). In neoplastic tissue: To determine at

what stage of the tumorigenic process NF-KB was upregulated in pancreatic ductal adenocarcinomas, an established scoring system that evaluates both the percent and intensity was utilized.

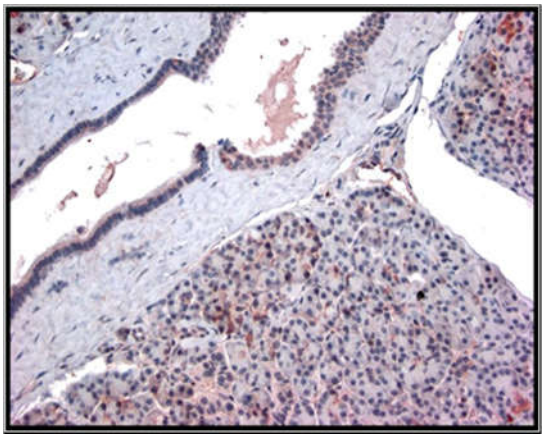
Ductal adenocarcinomas showed increased expression and activation of NF-kB. The staining was nuclear (p65) (Picture 3, 4) and cytoplasmic (p-IκBalpha) (Picture 5) respectively. Carcinoma samples showed NF-KB expression as score 0: 24, score 1:20, score 2: 27, score 3: 13, and score 4: 23 (Figure 1). With Spearman rank correlation, NF-κB(p65) expression had a correlation with lymph node status (p=0.04) (Table 1). No significant correlation was identified between NF-κB(p65), pIκBalpha expression and age, race, gender, location of the tumor, size of the tumor, margin status, tumor grade. None of the markers had a significant correlation with the overall survival (Figure 2).

DISCUSSION

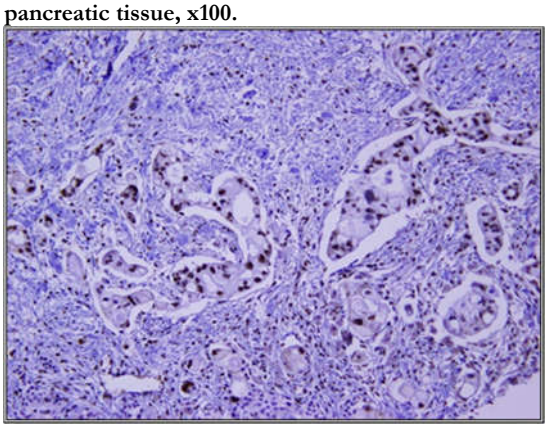
Pancreatic cancer is one of the most lethal cancers. Five-year-survival rate is 8%[1] Siegel et al. reported that in 2018 estimated number of pancreatic cancer diagnosis is approximately 55.440 and estimated number of death is 44.330[1]. In the developed countries, the incidence of PDA is 3.1-20.8 per 100.000[38]. It is commonly seen between the age 60 and 80, very rarely diagnosed before 40[39-42]. There is a male dominancy[42]. In this study, the mean age was 64. 66 were female and 41 were male.



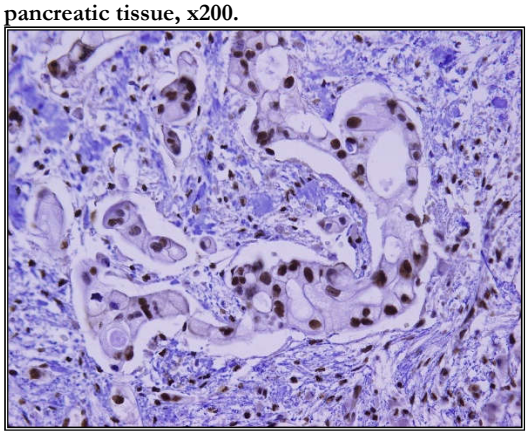
Picture 1. Expression of NF-kB(p65) in benign pancreatic tissue, x100.



Picture 2. Expression of fosfo-IKBa in benign pancreatic tissue, x200.



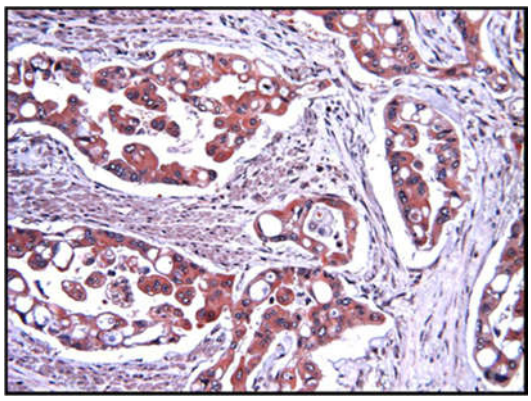
Picture 3. Nuclear staining for NF-kB (p65) in pancreatic ductal carcinoma, x200.



Picture 4. Nuclear staining for NF-kB (p65) in pancreatic ductal carcinoma, x400.

PDA mostly occurs in the head of the pancreas[43]. In the current study, 90 cases were located in the head of the pancreas while 17 were in the tail of the pancreas. The size of the PDA's are generally 2-5

cm[43]. In our study, the mean tumor size was 3.5 cm. In 84 cases, the tumor was larger than 2 cm. PDA tends to show early invasion to retroperitoneal tissue and local peripancreatic lymph nodes. Luttges et al reported 50% regional lymph node invasion and 10% paraaortic lymph node invasion[44]. In our study, there was lymph node metastasis in 25 cases out of 103.



Picture 6. Cytoplasmic staining for fosfo-IKBa in pancreatic ductal carcinoma, x400.

Even though surgery is a treatment option, the 5-year-survival is not more than 3 years in 80-90% cases[45-47]. In this study, the mean survival was 11 months. During this study, 18 patients died and 89 patients were alive. Identifying molecules of prognostic values would certainly help in understanding pancreatic carcinogenesis, elucidating clinicopathological variables of pancreatic tumors and improving survival of the patients.A key point present in pancreatic adenocarcinomas is the unique profile of genetic and molecular alterations that distinguishes it from all other cancers[48, 49].

Here we showed the clinicopathologic importance of NF-KB expression in PDA by using immunohistochemistrical analyses. Our data indicate that while normal pancreas showed weak staining in the ducts, and focally in the acini, there was an increased expression of NF-KB in the PDA, which is most likely an early event in pancreatic carcinogenesis.

NF-KB is a transcription factor, which comprises five subunits[8]: p65, c-Rel, Rel-B, NF-kB1 and NF-kB2[9-14]. Many reports demonstrated that members of the NF-KB family are involved in development of cancer. They are identified in many human hematopoietic malignancies and several types of solid tumor, such as human non-small cell lung carcinoma[50], squamous carcinomas of head and neck, and in adenocarcinomas of breast[23-26] and stomach[51], thyroid carcinoma cell lines[52], colon[21], prostate[23-25], bone and brain cancer cells[22] as well as pancreatic adenocarcinomas[35].

NF-KB activity is found constitutively activated in about 70% of pancreatic cancers[35]. It is consistent with our finding that NF-KB was weakly expressed in the ducts, and focally in the acini of the normal pancreas while ductal adenocarcinomas showed increased expression and activation of NF-KB.

Pham et al[53] investigated signal protein profile of PDA using tissue microarray. Cytoplasmic proteins such as p-JNK, p-ERK, p-SRC, p-NF-KB and nuclear proteins p-ERK, p-p38, p-JNK have higher activity in PDA than normal ductal epithelium. There is a significant increase in NF-KB levels in the lymph node metastasis. In the current study, similar to the Pham et al.'s study, there was an apparent relationship between lymph node status and the invasiveness of the tumor.

Weichert et al[54] reported the results of NF-KB p65 in 82 PDA, 5 chronic pancreatitis with the analysis of. clinicopathological correlation. There is no correlation with age and differantiation grade but there is higher cytoplasmic and nuclear NF-KB p65 positivity in the cases with lymph node metastasis. In our study, NF-KB expression levels were also correlated with margin and lymph node status, stage, tumor size, survival. Although, none of the parameters had significant correlation with NF-kB(p65) or pIkBalpha, a correlation with lymph node status (p=0.04) was identified which suggests a role in invasive properties of the tumor.

NF-KB is an important target due to the increase of the expression in pancreatic cancer for preventive and therapotic approaches[17]. Chemotherapy increases the survival in pancreatic cancer[55,56]. The study with gemcitabine in the late 1990's proved that data[55,56]. Gemcitabine is a chemotherapotic agent which has similar effect with 5-FU on pancreatic cancer. It has been discovered that gemcitabine has an advantageous place on survival and quality of life[55,56]. On the other hand there can be resistance to Gemcitabine treatment[57]. Therefore molecular targets are being investigated. NF-KB is activated in chemo resistant cell lineages hence among the therapeutic alternatives, it is one of the potential target molecules[57,58]. Particularly, curcumin is an important chemotherapeutic agent, which activates the apoptosis in pancreatic cancer and inhibits the

NF-KB activity[59]. This agent is still in the phase II studies in the MD Anderson researches[60]. In summary, despite the lack of an effect on overall survival, due to its increased activation in pancreatic cancer, NF-kB is still a good target for therapeutic interventions.

Yazar Katkıları: Çalışma konsepti/Tasarımı: DT, NÜB; Veri toplama: DT; Veri analizi ve yorumlama: DT., NÜB; Yazı taslağı: DT; İçeriğin eleştirel incelenmesi: NÜB; Son onay ve sorumluluk: DT, NÜB; Teknik ve malzeme desteği: DT; Süpervizyon: NÜB, DT; Fon sağlama (mevcut ise): yok.
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Author Contributions: Concept/Design : DT, NÜB; Data acquisition: DT; Data analysis and interpretation: DT, NÜB; Drafting manuscript: DT; Critical revision of manuscript: NÜB; Final approval and accountability: DT, NÜB; Technical or material support: DT; Supervision: NÜB, DT; Securing funding (if available): n/a.
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REFERENCES

1. Siegel R, Miller K, Jemal A. Cancer statistics 2018. CA Cancer J Clin. 2018;68:7-30.2.
2. Haller D. New perspectives in the management of pancreas cancer. Semin Oncol. 2003;30:3-10.
3. Hezel A, Kimmelman A, Stanger B, Bardeesy N, Depinho R. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev. 2006;20:1218-49.
4. Khan M, Azim S, Zubair H, Bhardwaj A, Patel G, Khushman M et al. Molecular Drivers of Pancreatic Cancer Pathogenesis: Looking Inward to Move Forward. Int J Mol Sci. 2017;18:779.
5. Ling J, Kang Y, Zhao R, Xia Q, Lee DF, Chang Z et al. KrasG12D-induced IKK2/β/NF-κB activation by IL-1α and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. Cancer Cell. 2012;21:105-20.
6. Prabhu L, Mundade R, Korc M, Loehrer P, Lu T. Critical role of NF-κB in pancreatic cancer. Oncotarget. 2014;5:22.
7. Tuncel D. Role of NF-Kappa B in the Approach to Pancreatic Ductal Adenocarcinoma. Archives Medical Review Journal. 2015;24:565-77.
8. Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. Oncogene. 2006;25:6680-4.
9. Verma IM, Stevenson JK, Schwarz EM, Van Antwerp D, Miyamoto S. Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. Genes Dev. 1995;9:2723-35.
10. Siebenlist U, Franzoso G, Brown K. Structure, regulation and function of NF-kappa B. Annu Rev Cell Biol, 1994;10:405-55.
11. Baldwin, A.S., Jr., The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol, 1996;14:649-83.
12. Ghosh S, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol. 1998;16:225-60.
13. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol. 2000;18:621-63.
14. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. Cell. 2002;109:81-96.
15. Sen R, Baltimore D. Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. Cell. 1986;47:921-8.
16. Luo JL, Kamata H, Karin M. IKK/NF-kappaB signaling: balancing life and death--a new approach to cancer therapy. J Clin Invest. 2005;115:2625-32.
17. Sarkar FH, Li YW. Targeting multiple signal pathways by chemopreventive agents for cancer prevention and therapy. Acta Pharmacol Sin. 2007;28:1305-15.
18. Sumitomo M, Tachibana M, Ozu C, Asakura H, Murai M, Hayakawa M et al. Induction of apoptosis of cytokine-producing bladder cancer cells by adenovirus-mediated IkappaBalpha overexpression. Hum Gene Ther. 1999;10:37-47.
19. Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM et al. Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer. J Clin Invest. 1997;100:2952-60.
20. Nakshatri H, Bhat-Nakshatri H, Martin DA, Goulet RJ, Sledge GW. Constitutive activation of NF-kappaB during progression of breast cancer to hormone-independent growth. Mol Cell Biol. 1997;17:3629-39.
21. Dejardin E, Deregowski V, Chapelier M, Jacops N, Gielen J, Merville MP et al. Regulation of NF-kappaB activity by I kappaB-related proteins in adenocarcinoma cells. Oncogene. 1999;18:2567-77.
22. Bours V, Dejardin E, Goujon-Letawe F, Merville MP, Castronovo V. The NF-kappa B transcription factor and cancer: high expression of NF-kappa B- and I kappa B-related proteins in tumor cell lines. Biochem Pharmacol. 1994;47:145-9.
23. Sumitomo M, Tachibana M, Nakashima J, Murai M, Miyajima A, Kimura F et al. An essential role for nuclear factor kappa B in preventing TNF-alpha-induced cell death in prostate cancer cells. J Urol. 1999;161:674-9.
24. Suh J, Payvandi F, Edelstein LC, Amenta PS, Zong WX, Gelinas C et al. Mechanisms of constitutive NF-kappaB activation in human prostate cancer cells. Prostate. 2002;52:183-200.
25. Herrmann JL, Beham AW, Sarkiss M, Chiao PJ, Rands MT, Bruckheimer EM et al. Bcl-2 suppresses apoptosis resulting from disruption of the NF-kappa B survival pathway. Exp Cell Res. 1997;237:101-9.

26. Devalaraja MN, Wang DZ, Ballard DW, Richmond A. Elevated constitutive IkappaB kinase activity and IkappaB-alpha phosphorylation in Hs294T melanoma cells lead to increased basal MGSA/GRO-alpha transcription. Cancer Res. 1999;59:1372-7.
27. Wang W, Abbruzzese JL, Evans DB, Larry L, Cleary KR, Chiao PJ. The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. Clin Cancer Res. 1999;5:119-27.
28. Sebens S, Arlt A, Schafer H. NF-kappaB as a molecular target in the therapy of pancreatic carcinoma. Recent Results Cancer Res. 2008;177:151-64.
29. Sclabas GM, Fujioka S, Schmidt C, Evans DB, Chiao PJ. NF-kappaB in pancreatic cancer. Int J Gastrointest Cancer, 2003;33:15-26.
30. Dong QG, Sclabas GM, Fujioka S, Schmidt C, Peng B, Wu T et al. The function of multiple IkappaB : NF-kappaB complexes in the resistance of cancer cells to Taxol-induced apoptosis. Oncogene. 2002;21:6510-9.
31. Yeh PY, Chuang SE, Yeh KH, Song YC, Ea CK, Cheng AL. Increase of the resistance of human cervical carcinoma cells to cisplatin by inhibition of the MEK to ERK signaling pathway partly via enhancement of anticancer drug-induced NF kappa B activation. Biochem Pharmacol. 2002;63:1423-30.
32. Mufson RA, Myers C, Turpin JA, Meltzer M. Phorbol ester reduces constitutive nuclear NF kappa B and inhibits HIV-1 production in mature human monocytic cells. J Leukoc Biol. 1992;52:637-44.
33. Slater AF, Kimland M, Jiang SA, Orreneus S. Constitutive nuclear NF kappa B/rel DNA-binding activity of rat thymocytes is increased by stimuli that promote apoptosis, but not inhibited by pyrrolidine dithiocarbamate. Biochem J. 1995;312:833-8.
34. Darieva ZA, Pospelov VA, Pospelova TV. Transcription factor NF-kappa B/RelA are constitutively activated and localized in the cell nuclei of E1A + cHa-Ras transformants. Tsitologiia. 1999;41:622-7.
35. Wang W, Abbruzzese JL, Evans DB, Chiao PJ. Overexpression of urokinase-type plasminogen activator in pancreatic adenocarcinoma is regulated by constitutively activated RelA. Oncogene. 1999;18:4554-63.
36. Yang J, Richmond A. Constitutive IkappaB kinase activity correlates with nuclear factor-kappaB activation in human melanoma cells. Cancer Res. 2001;61:4901-9.
37. Adsay NV, Basturk O, Bonnett M, Kilinc N, Andea AA, Feng J. A proposal for a new and more practical grading scheme for pancreatic ductal adenocarcinoma. Am J Surg Pathol. 2005;29:724-33.
38. Hruban R, Boffetta P, Hiraoka N, Iacobuzio-Donahue C, Kato Y, Kern SE et al. Ductal adenocarcinoma of the pancreas, in Pathology and genetics of tumors of the digestive system. WHO classification of the tumors of the Digestive System.Eds F. Bosman, F. Carneiro, R. Hruban, N. Theise. 2010, IARC Press: Lyon. p.281-291.
39. Blackbourne L, Jones R, Catalano C, Iezzoni J, Bourgeois F. Pancreatic adenocarcinoma in the pregnant patient: case report and review of the literature. Cancer. 1997;79:1776-9.
40. Luttges J, Stigge C, Pacena M, Kloppel G. Rare ductal adenocarcinoma of the pancreas in patients younger than age 40 years. Cancer. 2004;100:173-82.
41. Pernick N, Eldean Z, Kabbani W, Philip P, Shields A, Vaitkevicius V et al. Pancreatic ductal adenocarcinoma in young patients (abstract). Modern Pathology. 2001;14:201A.
42. Evans D, Abruzzese J, Rich T.Cancer of the pancreas. In Textbook of Cancer: Principles and practice of oncology.(Eds V.T. Devita, S. Hellman, S.A. Rosenberg) 1126-1161. Lippincott-Raven ,MA, 2001.
43. Brennan M, Moccia R, Klimstra D. Management of adenocarcinoma of the body and tail of the pancreas. Ann Surg. 1996; 223:506-11.
44. Luttges J, Vogel I, Menke M, Henne-Bruns D, Kremer B, Kloppel G. The retroperitoneal resection margin and vessel involvement are important factors determining survival after pancreaticoduodenectomy for ductal adenocarcinoma of the head of the pancreas. Virchows Arch. 1998;433:237-42.
45. Neoptolemos J, Stocken D, Friess H, Bassi C, Dunn J, Hickey H et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med. 2004;350:1200-10.
46. Nagakawa T, Nagamori M, Futakami F, Tsukioka Y, Kayahara M, Ohta T et a. Results of extensive surgery for pancreatic carcinoma. Cancer.1996;77:640-5.
47. Trede M, Schwall G, Saeger H. Survival after pancreatoduodenectomy. 118 consecutive resections without an operative mortality. Ann Surg.1990; 211:447-58.
48. Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. Clin Cancer Res, 2000;6:2969-72.
49. Hruban RH, Wilentz RE, Kern SE. Genetic progression in the pancreatic ducts. Am J Pathol. 2000;156:1821-5.
50. Mukhopadhyay T, Roth JA, Maxwell SA. Altered expression of the p50 subunit of the NF-kappa B transcription factor complex in non-small cell lung carcinoma. Oncogene. 1995;11:999-1003.
51. Motokura T, Arnold A. PRAD1/cyclin D1 proto-oncogene: genomic organization, 5' DNA sequence, and sequence of a tumor-specific rearrangement breakpoint. Genes Chromosomes Cancer. 1993;7:89-95.
52. Visconti R, Cerutti J, Battista S, Fedele M, Trapasso F, Zeki K et al. Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires

NFkappaB p65 protein expression. Oncogene. 1997;15:1987-94.
53. Pham N, Schwock J, Iakovlev V, Pond G, Hedley D, Tsao M. Immunohistochemical analysis of changes in signaling pathway activation downstream of growth factor receptors in pancreatic duct cell carcinogenesis. BMC Cancer. 2008;8:43.
54. Weichert W, Boehm M, Gekeler V, Bahra M, Langrehr J, Neuhaus P et al. High expression of RelA/p65 is associated with activation of nuclear factor-kappaB-dependent signaling in pancreatic cancer and marks a patient population with poor prognosis. Br J Cancer. 2007;97:523-30.
55. Burris H, Storniolo A. Assessing clinical benefit in the treatment of pancreas cancer: gemcitabine compared to 5-fluorouracil. Eur J Cancer. 1997;33:18-22.
56. Burris H, Moore M, Andersen J, Green M, Rothenberg M, Modiano M et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol. 1997;15:2403-13.
57. Arlt A, Gehrz A, Muerkoster S, Vorndamm J, Kruse M, Folsch U et al. Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. Oncogene. 2003;22:3243-51.
58. Chandler N, Canete J, Callery M. Increased expression of NF-kappa B subunits in human pancreatic cancer cells. J Surg Res. 2004;118:9-14.
59. Li L, Braiteh F, Kurzrock R. Liposome-encapsulated curcumin: in vitro and in vivo effects on proliferation, apoptosis, signaling, and angiogenesis. Cancer. 2005;104:1322-31.
60. Dhillon N, Aggarwal BB, Newman RA, Wolff RA, Kunnumakkara AB, Abbruzzese JL et al. Phase II trial of curcumin in patients with advanced pancreatic cancer. Clin Cancer Res. 2008;14:4491-9.