Transcription factors: Are they a real target for future therapeutic strategies?

Michael Roth

The Woolcock Institute of Medical Research, Camperdown, NSW-2050, Australia and Pulmonary Cell Research, Pneumology, University Hospital Basel, CH-4031 Basel, Switzerland.

Introduction

Transcription factors have been identified as the major regulators of gene activity and therefore control the expression of most known proteins. Transcription factors *per se* do not exert any other function than to transform a message that was received by a cell via a receptor and its subsequent signalling molecules into the nucleus and onto the level of gene activity by either promoting or silencing gene transcription (1, 2).

The first transcription factors characterised had been often linked to malignant transformation and where e.g. termed “onco-genes” (3, 4), a misleading term as we know today. Today we have learned that transcription factors can be activated by most known growth factors and cytokines but also by hypoxia or oxidative stress (5, 6), lipids and polyunsaturated fatty acids (7), light (8, 9), stress (10, 11), or mechanical forces such as stretching (12, 13), as a response the expression of a whole array of genes is modified. Out of the function of certain transcription factors came the idea that if one can control their activity transcription factor modulation would be a tool to cure diseases (14-19). Such generalised conclusions are dangerous and ignore several basic aspects of cell biology.

The usual transcription factor resides in an inert inactive conformation associated to the cell membrane, the endoplasmatic reticulum, or at a less well characterised localisation in the cytosol. Upon activation a transcription factor undergoes a conformational change and attaches to specific carrier proteins that translocate it into the nucleus where it recognises a more or less specific DNA sequence to which it binds. Binding onto it specific DNA sequence affects the structural conformation of the DNA sequence and of some of the neighbouring nucleotides, the DNA un- or up-winds and becomes more accessible for binding of the RNA transcription machinery if the transcription factor activates a gene. However, transcriptional

regulation is dependent not only on transcription factor activation and chromatin remodeling, but also on co-regulators, co-activators, and co-repressors of the transcription factors.

Furthermore, transcription factors can only exert their function if they find a specific the DNA sequence and folding pattern that has to be associated with the right chromatin formation. There is an expanding array of additional modifications that titrate transcriptional regulation for the specific conditions of a particular cell type, organ system, and developmental stage, and such events are likely to be greatly influenced by upstream signalling cascades (1, 2, 20). While the basic mechanism as the proximal binding of essential transcription factors to the TATA-box and their interaction with the RNA polymerase II and co-activators has been studied for several transcription factors their function as a co-activator or transcription enhancer factor binding at a more distant location to the TATA-box is not well understood and is dependent on the folding and the relative location of the binding site to the TATA-box binding transcription factor (2, 21). A similar scenario accounts for the silencing function of transcription factors, where a single or multiple transcription factors bind to a defined DNA sequence and inhibit the action of the transcription machinery, e.g. the glucocorticoid receptor often silences genes which have no classical glucocorticoid response element in their promoter (22, 23).

Cell type specific transcription factors:

Beside general occurring and functioning transcription factors there are some cell type specific ones and those that exert a specific function under specific conditions or according in which cell type they are activated (24). Transcription factors Pax3 induced a mesenchymal to epithelial transition and the Pax3/FKHR complex affected the morphogenesis of human osteosarcoma cells (25). LEF1 affects cell survival in epithelial cells during tooth development were it mediates the Wnt signal (26). Several trophoblast specific transcription

factors have been reviewed by Knofler et al (27) but those have been mainly described in mouse models. However, since not all transcription factors have been identified yet “cell type specific” has to be used with caution as shown by a review regarding “organ specific” transcription factors including forkhead box A2 (HNF)-3beta, HNF-3/forkhead homolog-8 (FoxF1), HNF-3/forkhead homolog-4 (FoxJ1), thyroid transcription factor-1 (Nkx2.1), homeodomain box A5 transcription factor, the zinc finger protein Gli, and GATA transcription factors, as well as the basic helix-loop-helix Pod1 transcription factor (28). The family of MyoD transcription factors was described as myogenic differentiation factor triggering the maturation of smooth muscle cells (29). However, MyoD alone seems to be insufficient to control smooth muscle cell differentiation and needs the interaction with other related transcription factors (30). Therefore, it is often misleading to term a transcription factor cell type specific and more research is needed.

How to modify the action of transcription factors in therapy:

In order to use a transcription factor as a target in a new therapeutic strategy it seems to be unwise to block its function in an entire organism or to silence it for extended periods. There are several options to modify the action of transcription factors: (i) direct blocking of their activation by specific drugs, (ii) suppression of their expression by antisense oligo nucleotides, (iii) expression control by small inhibitory RNAs (siRNA), (iv) “catching away” activated transcription factors with decoy DNA oligo nucleotides of a specific DNA-binding sequence, (v) intercepting their interaction with other proteins, or (vi) transfection diseased organs/cells with overexpression vectors for a missing transcription factor. An overview of the current available methods that can be used to control the expression or function of transcription factors is provided in the following:

Antisense oligodeoxynucleotides can be used to induce a fast degradation of any gene of interest and use a natural occurring mechanism of the cells defence system based on the activation of RNase H that recognizes double stranded RNA-DNA strands and cleaves them (31). Most of the approaches to down regulate the expression of transcription factors by antisense oligonucleotides are in a trial phase which did not exceed the cell culture level or animal model. A major problem for antisense oligonucleotides is the delivery system. Antisense oligonucleotides are sensitive to a variety of enzymes present in the blood stream and in other body fluids (31 - 33).

Small inhibitory RNAs also use a natural occurring cellular virus defense system. Here a double stranded short (20-40 nucleotides) oligodesoxyribonucleotide sequence is introduced into the cell where it is recognised by specific enzymes. The double stranded RNA is bound, one of ist strands is degraded and the the remaining strand is used by the enzyme to find a new matching single stranded mRNA which binds and is degraded. Intersstingly the detecting RNA strand is not degraded by the enzyme and is re-used to find and destroy traget mRNAs (34, 35). The mRNA eliminating effect of siRNA seem to be very precise and target specific, however, the latter effect depends on the carefull design of the to be used sequence. Similar problematic is the cell type specific delivery.

A more promising method was termed decoy oligonucleotides. Decoy oligonucleotides are synthetic double stranded oligonucleotides containing a transcription factor specific DNA consensus sequence and if present in the cytosol of any cell will bind active transcription factors and thereby prevent their migration into the nucleus. This method does not down-regulate the expression of a transcription factor but interferes with its function by compeating with its natural target DNA sequence in the nucleus. Decoy oligonucleotides have been successfully used to suppress the action of e.g. nuclear factor-kappaB (NF-kappaB) and E2F to prevent the progression of several diseases including renal diseases (36). Tdecoy

oligonucleotides have been successfully used to control the activity of the activator protein-1 (AP-1), negative regulatory element (NRE) for the renin gene and angiotensinogen gene-activating element (AGE), and ets-1 (37 - 40). .

A major problem using any oligonucleotide construct is its short bio-stability, chemical modification in order to increase nuclease resistance often are accompanied by a decrease in the affinity for to the transcription factor or a loss of specificity of transcription factor binding. Neither circular dumbbell, nor chimeric decoy oligonucleotides changed the sensitivity to endonuclease cleavage. To address these problem so called “locked nucleic acids” of a decoy oligonucleotide for NF-kappaB have been designed with an increased stability compared to end-capped oligonucleotide (41). Such modified PNA-DNA are less sensitive to degrading enzymes and are still efficiently recognized by e.g. NF-kappaB, but their design and their safety in long term use as a drug has to proven (41 - 44).

Peptide nucleic acids-DNA chimeras, defined as a double stranded DNA sequence molecules that binds to transcription factors in a sequence-dependent manner. An example has been described for NF-kappaB and Sp1 and were used in vitro cultured in human cells and there efficacy was found to be comparable to those observed using double-stranded DNA decoys (45 - 47). The advantage of this method may be that based on peptide nucleic acids -DNA chimeras can be modified by the addition of short peptides that ease cell penetration and nuclear localization. This option may also hold the key to a cell type specific delivery of oligonucleotides.

Transcription factors as a traget in therapy:

Activator protein-1 (AP-1): AP-1 controls proliferation and cytokine expression and therefore is invovled in tumorigenesis and in inflammatory diseases. Together with histone

acetyltransferase CBP/p300 and the transcription factors CCAAT/enhancer binding protein (C/EBP), nuclear factor – AT (NF-AT) AP-1 controls the expression of the asthma associated cytokine IL-5 and can be activated also by rhinvirus infection (48 - 51). AP-1 can be blocked by either suppression of its activation or by inhibition of AP-1 binding to its DNA consensus sequence. Both methods concepts have been successfully used in animal and cell culture models but not yet in patients. A small-molecule AP-1 inhibitor, PNRI-299, targets the oxidoreductase, redox effector factor-1, was reported in human lung epithelial cells (IC_{50}: 20 microM) and reduced eosinophil infiltration in mice (52). MOL 294 blocks AP-1 and NF_KB inhibitor, controlling both factors at the transcriptional level (52). SP100030 inhibited AP-1 and NF_KB activation in Brown-Norway rats (53). Cyclosporin A, the adrenal steroid dehydroepiandrosterone, and the macrolide clarithromycin suppress DNA-binding of AP-1 (37, 54, 55). Decoy oligonucleotides have also been successfully used to block the action of AP-1 (35).

The CCAAT/enhancer binding proteins (C/EBP): Human C/EBPs consist of six known isoproteins (-α, -β, -γ –δ, -ε, and –ζ) which are all keep the balance between cell proliferation and cell differentiation and may even be expressed in a cell type specific pattern in the lung (56 – 58). The hyperplasia of smooth muscle bundles in the airways of patients with asthma seem to originate from a lack of C/EBP- α that leads to a less good growth control in this cells (59). Ceramide, a sphingolipid, inhibits the transport of activated C/EBP-α and NF-E2-related factor-2 (Nrf2) into the nucleus (60). Curcumine interfere with C/EBP-α and –β binding to their DNA consensus sequence (61). Trichostatin A inhibits C/EBP-α mRNA levels while valproic acid down-regulated its protein expression in adipocytes (62). Good ccontrol of smooth muscle cell differentiation was achieved by CCAAT-decoy oligonucleotide and were well tolerated in vivo in animal models and in pre-clinical studies (63 - 65). However, a

general blockade of all C/EBP-isoproteins by e.g. decoy oligonucleotides is not advisable since the various C/EBP-isoproteins exert distinct often opposing function (65). Here only cell type specific inhibition or induction of C/EBPs will help and has to be further investigated.

GATA-: GATA-3 a cell lineage-specific factor expressed in Th2 cells subsequent to STAT-6 activation in the context of inflaamtory diseases (66, 67). GATA-3 function was sucessfully suppressed by antisense oligonucleotides (68).

Nuclear Factor$_{kappa}$B (NF$_K$B): The NF$_K$B transcription factor family is involved in the pathology of many diseases including AIDS, atherosclerosis, arthritis, asthma, cancer, diabetes, inflammatory bowel disease, muscular dystrophy, stroke, and viral infections (66, 67). Its ubiquitous action is important in host defence and in chronic inflammatory diseases and therefore, NF$_K$B control was early suggested as a potential therapeutic approach for (69, 70). Glucocorticoids have been reported to inhibit NF$_K$B activity but the mechanism is unclear (71 - 74). Pranlukast, pyrrolydine dithiocarbamate, and fosfomycin have been reported to inhibit NF$_K$B activity (74 - 76). Isoprenoids such as the kaurene diterpenoid and sesquiterpene lactone class and also the flavonoid silybin inhibit NF$_K$B activity, but their mode of action his unknown (77, 78). In an animal model decoy oligodeoxynucleotides NF$_K$B blocked its activity efficiently in airway immune cells, but not in structural lung cells which may indicate a cdll type specific delivery mode opf decoy oligonucleotides (77, 78).

Nuclear factor E2 p45-related factor 2 (*Nrf2)/ antioxidant response element (ARE*): Nrf2/ARE is involved in the oxidative stress response in a beneficial manner that would protect hte affected cell from stress and therefore not inhibition but activation wopuld be of interest for therapy (79 - 81). As for C/EBPs ceramide inhibited the transport of activated

Nrf2 into the nucleus (60) which indicates ist low transcription factor specificity. Moreover, if cermaide would be used to block the pro-inflammatory action of NF_KB at the same time it would block the benficial action of Nrf2/ARE.

Peroxisome Proliferator-activated Receptor (PPAR): PPAR (-α, -β, -γ, -δ) interact with the glucocorticoid receptor and with several C/EBP-isoproteins and therefore participate in cell proliferation and differentiation. They therefore have a similar critical role in the pathogenesis of several diseases (82, 83). PPARγ agonists, glucocorticoids, and β_2-agonists synergistically suppress cytokine expression at the transcriptional level (83). In regard to therapy there are already specific PPAR-isoform inhibitors and activators available (84). 15-deoxy-Delta(12,14)-PGJ(2) (15d-PGJ(2)) and troglitazone both activate PPARγ inhibited the pro-inflammatory action of TNFα (85). Ciglitazone and GW9662, two other PPARα agonists decreased airway hyperresponsiveness, basement membrane thickness, mucus production, collagen deposition, and TGFα synthesis in a mouse model (85, 86). PPAR-δ, incontrast, acts as an pro-inflammatory factor and binds to the same target DNA sequencess as PPARα, –γ, and its enhanced expression would be no benefit for asthma (82, 84 - 86). However, there could be a cell type or species specific effect of PPARs action since under certain conditions troglitazone counteracts the inhibitory effect of rapamycin on the PPARγ-C/EBP-α interaction (87).

The signal transducer and activator of transcription 6 (STAT6): The role of the JAK-STAT system in health and inflammatory lung diseases was reviewd by Pernis and Rothman 2002 (88). TNFα, TGF-β, IL-4, and IL-13 are known activators of STAT6 (89, 90), and a polymorphism in the human STAT6 promoter could explain the genetic pre-disposition of high IgE levels in asthma patients and their siblings (91). Up to date the action of STAT6 is

only known to be inhibited by glucocorticoids (92, 93) and no other inhibitors for STAT6 have so far been reported.

Conclusion

Transcription factors are important regulators for immune response, inflammation and tumorigenesis their inhibition or stimulation may cure a disease without the side effects of standard drugs if they can be applied in a cell type specific mode, but a practical application of the bench work that has been done may take some more years. A major point of concern is that most transcription factors are central control factors of tissue and organ homeostasis and each longterm inhibition or activation will most likely cause severe side effects in other organs. Cell type specific application of decoy or antisense oligonucleotides for NF_KB, Nrf2 or STAT6, or specific agonists for PPARα and $-\gamma$ have been attempted but not yet proven to be harmless over long period application. The key to success with such anovel therapeutic approach using transcription factors as medication comes with the cell type specific delivery system, a well known and not yet solved problem with the existing drugs.

References:

1. Stamatoyannopoulos JA. The genomics of gene expression. Genomics 2004;84:449-57.
2. Villard J. Transcription regulation and human diseases. Swiss Med Wkly 2004;134:571-9.
3. Racker E. Oncogenes, transforming growth factors, and protein transport: a hypothesis. Biosci Rep 1983;3:507-16.
4. Cherkaoui Malki M, Lone YC, Corral-Debrinski M, Latruffe N. Differential proto-oncogene mRNA induction from rats treated with peroxisome proliferators. Biochem Biophys Res Commun 1990;173:855-61.

5. Kabe Y, Ando K, Hirao S, Yoshida M, Handa H. Redox regulation of NF-kappaB activation: distinct redox regulation between the cytoplasm and the nucleus. Antioxid Redox Signal 2005;7:395-403.

6. Greijer AE, van der Wall E. The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. J Clin Pathol 2004;57:1009-14.

7. Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of gene expression. Nutr Rev 2004;62:333-9.

8. Ronai ZA, Okin E, Weinstein IB. Ultraviolet light induces the expression of oncogenes in rat fibroblast and human keratinocyte cells. Oncogene 1988;2:201-4.

9. Tanos T, Marinissen MJ, Coluccio Leskow F, Hochbaum D, Martinetto H, Gutkind JS, Coso OA. Phosphorylation of c-Fos by members of the p38 MAPK family: Role in the AP-1 response to UV. J Biol Chem 2005 Feb 10; epub

10. Bierhaus A, Humpert PM, Nawroth PP. NF-kappaB as a molecular link between psychosocial stress and organ dysfunction. Pediatr Nephrol 2004;19:1189-91.

11. Sabban EL, Hebert MA, Liu X, Nankova B, Serova L. Differential effects of stress on gene transcription factors in catecholaminergic systems. Ann N Y Acad Sci 2004;1032:130-40.

12. Komuro I, Kaida T, Shibazaki Y, Kurabayashi M, Katoh Y, Hoh E, Takaku F, Yazaki Y. Stretching cardiac myocytes stimulates protooncogene expression. J Biol Chem 1990;265:3595-8.

13. Inoue D, Kido S, Matsumoto T. Transcriptional induction of FosB/DeltaFosB gene by mechanical stress in osteoblasts. J Biol Chem 2004;279:49795-803.

14. Barnes PJ, Adcock IM. Transcription factors and asthma. Eur Respir J 1998;12:221-34.

15. Di Stefano A, Caramori G, Oates T, Capelli A, Lusuardi M, Gnemmi I, Ioli F, Chung KF, Donner CF, Barnes PJ, Adcock IM. Increased expression of nuclear factor-kappaB in bronchial biopsies from smokers and patients with COPD. Eur Respir J. 2002;20:556-63.

16. Katsuta M, Miyashita M, Makino H, Nomura T, Shinji S, Yamashita K, Tajiri T, Kudo M, Ishiwata T, Naito Z. Correlation of hypoxia inducible factor-1alpha with lymphatic metastasis via vascular endothelial growth factor-C in human esophageal cancer. Exp Mol Pathol 2005;78:123-30.

17. Ukon K, Tanimoto K, Shimokuni T, Noguchi T, Hiyama K, Tsujimoto H, Fukushima M, Toge T, Nishiyama M. Activator protein accelerates dihydropyrimidine dehydrogenase gene transcription in cancer cells. Cancer Res 2005;65:1055-62.

18. Toyama T, Nakamura H, Harano Y, Yamauchi N, Morita A, Kirishima T, Minami M, Itoh Y, Okanoue T. PPARalpha ligands activate antioxidant enzymes and suppress hepatic fibrosis in rats. Biochem Biophys Res Commun 2004;324:697-704.

19. Harja E, Bucciarelli LG, Lu Y, Stern DM, Zou YS, Schmidt AM, Yan SF. Early growth response-1 promotes atherogenesis: mice deficient in early growth response-1 and apolipoprotein E display decreased atherosclerosis and vascular inflammation. Circ Res 2004;94:333-9.

20. A. Kumar R, Wang RA, Barnes CJ. Coregulators and chromatin remodeling in transcriptional control. Mol Carcinog 2004;41:221-30.

21. Torres-Padilla ME, Weiss MC. Effects of interactions of hepatocyte nuclear factor 4alpha isoforms with coactivators and corepressors are promoter-specific. FEBS Lett 2003;539: 19-23.

22. De Martino MU, Bhattachryya N, Alesci S, Ichijo T, Chrousos GP, Kino T. The glucocorticoid receptor and the orphan nuclear receptor chicken ovalbumin upstream

promoter-transcription factor II interact with and mutually affect each other's transcriptional activities: implications for intermediary metabolism. Mol Endocrinol 2004;18:820-33.

23. Szapary D, Huang Y, Simons SS Jr. Opposing effects of corepressor and coactivators in determining the dose-response curve of agonists, and residual agonist activity of antagonists, for glucocorticoid receptor-regulated gene expression. Mol Endocrinol 1999;13:2108-21.

24. Yang J, Riley M, Thomas K. Cell-type-specific transcription factor interactions with cis-elements present in the mouse LDH/C proximal promoter region. J Exp Zool 1998;282: 179-87.

25. Begum S, Emani N, Cheung A, Wilkins O, Der S, Hamel PA. Cell-type-specific regulation of distinct sets of gene targets by Pax3 and Pax3/FKHR. Oncogene 2005 Jan 31; epub

26. Sasaki T, Ito Y, Xu X, Han J, Bringas P Jr, Maeda T, Slavkin HC, Grosschedl R, Chai Y. LEF1 is a critical epithelial survival factor during tooth morphogenesis. Dev Biol 2005; 278:130-43.

27. Knofler M, Vasicek R, Schreiber M. Key regulatory transcription factors involved in placental trophoblast development-a review. Placenta 2001;22 Suppl A:S83-92.

28. Costa RH, Kalinichenko VV, Lim L. Transcription factors in mouse lung development and function. Am J Physiol Lung Cell Mol Physiol 2001;280:L823-38.

29. Li L, Olson EN. Regulation of muscle cell growth and differentiation by the MyoD family of helix-loop-helix proteins. Adv Cancer Res 1992;58:95-119.

30. Fux C, Langer D, Fussenegger M. Dual-regulated myoD- and msx1-based interventions in C2C12-derived cells enable precise myogenic/osteogenic/adipogenic lineage control. J Gene Med. 2004;6:1159-69.

31. Galarneau A, Min KL, Mangos MM, Damha MJ. Assay for evaluating ribonuclease H-mediated degradation of RNA-antisense oligonucleotide duplexes. Methods Mol Biol 2005;288:65-80.

32. Wang JY, Drlica K. Computational identification of antisense oligonucleotides that rapidly hybridize to RNA. Oligonucleotides 2004;14:167-75.

33. Opalinska JB, Kalota A, Gifford LK, Lu P, Jen KY, Pradeepkumar PI, Barman J, Kim TK, Swider CR, Chattopadhyaya J, Gewirtz AM. Oxetane modified, conformationally constrained, antisense oligodeoxyribonucleotides function efficiently as gene silencing molecules. Nucleic Acids Res 2004;32:5791-9.

34. Nesterova M, Cho-Chung YS. Killing the messenger: antisense DNA and siRNA. Curr Drug Targets 2004;5:683-9.

35. Heale BS, Soifer HS, Bowers C, Rossi JJ. siRNA target site secondary structure predictions using local stable substructures. Nucleic Acids Res 2005 Feb;33:epub.

36. Morishita R, Tomita N, Kaneda Y, Ogihara T. Molecular therapy to inhibit NFkappaB activation by transcription factor decoy oligonucleotides. Curr Opin Pharmacol. 2004;4:139-46.

37. Ahn JD, Morishita R, Kaneda Y, Lee SJ, Kwon KY, Choi SY, Lee KU, Park JY, Moon IJ, Park JG, Yoshizumi M, Ouchi Y, Lee IK. Inhibitory effects of novel AP-1 decoy oligodeoxynucleotides on vascular smooth muscle cell proliferation in vitro and neointimal formation in vivo. Circ Res. 2002;90:1325-32.

38. Tomita S, Tomita N, Yamada T, Zhang L, Kaneda Y, Morishita R, Ogihara T, Dzau VJ, Horiuchi M. Transcription factor decoy to study the molecular mechanism of negative regulation of renin gene expression in the liver in vivo. Circ Res 1999;84:1059-66.

39. Nishii T, Moriguchi A, Morishita R, Yamada K, Nakamura S, Tomita N, Kaneda Y, Fukamizu A, Mikami H, Higaki J, Ogihara T. Angiotensinogen gene-activating elements regulate blood pressure in the brain. Circ Res 1999 ;85:257-63.

40. Nakashima H, Aoki M, Miyake T, Kawasaki T, Iwai M, Jo N, Oishi M, Kataoka K, Ohgi S, Ogihara T, Kaneda Y, Morishita R. Inhibition of experimental abdominal aortic aneurysm in the rat by use of decoy oligodeoxynucleotides suppressing activity of nuclear factor kappaB and ets transcription factors. Circulation 2004;109:132-8.

41. Jepsen JS, Wengel J. LNA-antisense rivals siRNA for gene silencing. Curr Opin Drug Discov Devel 2004;:188-94.

42. Fisher L, Soomets U, Cortes Toro V, Chilton L, Jiang Y, Langel U, Iverfeldt K. Cellular delivery of a double-stranded oligonucleotide NFkappaB decoy by hybridization to complementary PNA linked to a cell-penetrating peptide. Gene Ther 2004;11:1264-72.

43. Pfundheller HM, Sorensen AM, Lomholt C, Johansen AM, Koch T, Wengel J. Locked nucleic acid synthesis. Methods Mol Biol 2005;288:127-46.

44. Filichev VV, Christensen UB, Pedersen EB, Babu BR, Wengel J. Locked nucleic acids and intercalating nucleic acids in the design of easily denaturing nucleic acids: thermal stability studies. Chembiochem. 2004;5:1673-9.

45. Romanelli A, Pedone C, Saviano M, Bianchi N, Borgatti M, Mischiati C, Gambari R. Molecular interactions with nuclear factor kappaB (NF-kappaB) transcription factors of a PNA-DNA chimera mimicking NF-kappaB binding sites. Eur J Biochem 2001;268:6066-75.

46. Borgatti M, Romanelli A, Saviano M, Pedone C, Lampronti I, Breda L, Nastruzzi C, Bianchi N, Mischiati C, Gambari R.Resistance of decoy PNA-DNA chimeras to enzymatic degradation in cellular extracts and serum. Oncol Res 2003;13:279-87.

47. Borgatti M, Lampronti I, Romanelli A, Pedone C, Saviano M, Bianchi N, Mischiati C, Gambari R. Transcription factor decoy molecules based on a peptide nucleic acid (PNA)-DNA chimera mimicking Sp1 binding sites. J Biol Chem 2003;278:7500-9.

48. Nguyen C, Teo JL, Matsuda A, Eguchi M, Chi EY, Henderson WR Jr, Kahn M. Chemogenomic identification of Ref-1/AP-1 as a therapeutic target for asthma. Proc Natl Acad Sci U S A. 2003;100:1169-73.

49. Liu C, Lu J, Tan J, Li L, Huang B. Human interleukin-5 expression is synergistically regulated by histone acetyltransferase CBP/p300 and transcription factors C/EBP, NF-AT and AP-1. Cytokine. 2004;27:93-100.

50. Ogawa K, Kaminuma O, Okudaira H, Kikkawa H, Ikezawa K, Sakurai N, Mori A. Transcriptional regulation of the IL-5 gene in peripheral T cells of asthmatic patients. Clin Exp Immunol. 2002;130:475-83.

51. Funkhouser AW, Kang JA, Tan A, Li J, Zhou L, Abe MK, Solway J, Hershenson MB. Rhinovirus 16 3C protease induces interleukin-8 and granulocyte-macrophage colony-stimulating factor expression in human bronchial epithelial cells. Pediatr Res. 2004;55:13-8

52. Henderson WR Jr, Chi EY, Teo JL, Nguyen C, Kahn M. A small molecule inhibitor of redox- regulated NF-kappa B and activator protein-1 transcription blocks allergic airway inflammation in a mouse asthma model.J Immunol. 2002;169:5294-9.

53. Huang TJ, Adcock IM, Chung KF. A novel transcription factor inhibitor, SP100030, inhibits cytokine gene expression, but not airway eosinophilia or hyperresponsiveness in sensitized and allergen-exposed rat. Br J Pharmacol. 2001;134:1029-36.

54. Abe S, Nakamura H, Inoue S, Takeda H, Saito H, Kato S, Mukaida N, Matsushima K, Tomoike H. Interleukin-8 gene repression by clarithromycin is mediated by the activator protein-1 binding site in human bronchial epithelial cells. Am J Respir Cell Mol Biol. 2000; 22:51-60.

55. Dashtaki R, Whorton AR, Murphy TM, Chitano P, Reed W, Kennedy TP. Dehydroepiandrosterone and analogs inhibit DNA binding of AP-1 and airway smooth muscle proliferation. J Pharmacol Exp Ther. 1998;285:876-83.

56. Cassel TN, Nord M C/EBP transcription factors in the lung epithelium. Am J Physiol Lung Cell Mol Physiol. 2003;285:L773-81.

57. Hu B, Wu Z, Jin H, Hashimoto N, Liu T, Phan SH. CCAAT/enhancer-binding protein beta isoforms and the regulation of alpha-smooth muscle actin gene expression by IL-1 beta. J Immunol. 2004;173:4661-8.

58. Takeji M, Kawada N, Moriyama T, Nagatoya K, Oseto S, Akira S, Hori M, Imai E, Miwa T. CCAAT/Enhancer-binding protein delta contributes to myofibroblast transdifferentiation and renal disease progression. J Am Soc Nephrol. 2004;15:2383-90.

59. Roth M, Johnson PR, Borger P, Bihl MP, Rudiger JJ, King GG, Ge Q, Hostettler K, Burgess JK, Black JL, Tamm M.: Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. N Engl J Med. 2004; 351:560-574.

60. Park IN, Cho IJ, Kim SG. Ceramide negatively regulates glutathione S-transferase gene transactivation via repression of hepatic nuclear factor-1 that is degraded by the ubiquitin proteasome system. Mol Pharmacol. 2004;65:1475-84.

61. Balasubramanian S, Eckert RL. Green tea polyphenol and curcumin inversely regulate human involucrin promoter activity via opposing effects on CCAAT/enhancer-binding protein function. J Biol Chem. 2004;279:24007-14.

62. Lagace DC, Nachtigal MW. Inhibition of histone deacetylase activity by valproic acid blocks adipogenesis. J Biol Chem. 2004;279:18851-60

63. Kelkenberg U, Wagner AH, Sarhaddar J, Hecker M, von der Leyen HE. CCAAT/enhancer- binding protein decoy oligodeoxynucleotide inhibition of macrophage-rich vascular lesion formation in hypercholesterolemic rabbits. Arterioscler Thromb Vasc Biol. 2002;22:949-54.

64. Wagner AH, Krzesz R, Gao D, Schroeder C, Cattaruzza M, Hecker M. Decoy oligodeoxynucleotide characterization of transcription factors controlling endothelin-B receptor expression in vascular smooth muscle cells. Mol Pharmacol. 2000;58:1333-40.

65. Quarcoo D, Hamelmann E. Transcription factors: new targets for antiallergic therapy. Pathobiology. 2002-2003;70:293-6.

66. Taha R, Hamid Q, Cameron L, Olivenstein R. T helper type 2 cytokine receptors and associated transcription factors GATA-3, c-MAF, and signal transducer and activator of transcription factor- 6 in induced sputum of atopic asthmatic patients. Chest. 2003;123:2074-82.

67. Caramori G, Lim S, Ito K, Tomita K, Oates T, Jazrawi E, Chung KF, Barnes PJ, Adcock IM. Expression of GATA family of transcription factors in T-cells, monocytes and bronchial biopsies. Eur Respir J. 2001;18:466-73.

68. Finotto S, De Sanctis GT, Lehr HA, Herz U, Buerke M, Schipp M, Bartsch B, Atreya R, Schmitt E, Galle PR, Renz H, Neurath MF Treatment of allergic airway inflammation and hyperresponsiveness by antisense-induced local blockade of GATA-3 expression. J Exp Med. 2001;193:1247-60.

69. Kumar A, Takada Y, Boriek AM, Aggarwal BB. Nuclear factor-kappaB: its role in health and disease. J Mol Med. 2004;82:434-48.

70. Yamamoto Y, Gaynor RB. IkappaB kinases: key regulators of the NF-kappaB pathway.Trends Biochem Sci. 2004;29:72-9.

71. De Boesscher K, Vanden Berghe W, Haegeman G. The interplay between the gluccorticoid receptor and Nuclear factor-KB or activator protein-1: Molecular Mechanisms for gene repression. Endo. Rev. 2003; 24: 488-522.

72. Wright JG, Christman JW. The role of nuclear factor kappa B in the pathogenesis of pulmonary diseases: implications for therapy. Am J Respir Med. 2003;2:211-9

73. Kagoshima M, Ito K, Cosio B, Adcock IM. Glucocorticoid suppression of nuclear factor-kappa B: a role for histone modifications. Biochem Soc Trans. 2003;31(Pt 1):60-5

74. Tomari S, Matsuse H, Machida I, Kondo Y, Kawano T, Obase Y, Fukushima C, Shimoda T, Kohno S. Pranlukast, a cysteinyl leukotriene receptor 1 antagonist, attenuates allergen-specific tumour necrosis factor alpha production and nuclear factor kappa B nuclear translocation in peripheral blood monocytes from atopic asthmatics. Clin Exp Allergy. 2003;33:795-801.

75. Ichiyama T, Hasegawa S, Umeda M, Terai K, Matsubara T, Furukawa S. Pranlukast inhibits NF- kappa B activation in human monocytes/macrophages and T cells. Clin Exp Allergy. 2003;33:802-7.

76. Yoneshima Y, Ichiyama T, Ayukawa H, Matsubara T, Furukawa S. Fosfomycin inhibits NF- kappaB activation in U-937 and Jurkat cells. Int J Antimicrob Agents. 2003;21:589-92.

77. Wuyts WA, Vanaudenaerde BM, Dupont LJ, Demedts MG, Verleden GM. Involvement of p38 MAPK, JNK, p42/p44 ERK and NF-kappaB in IL-1beta-induced chemokine release in human airway smooth muscle cells. Respir Med. 2003;97:811-7.

78. Bremner P, Heinrich M. Natural products as targeted modulators of the nuclear factor-kappaB pathway. J Pharm Pharmacol. 2002;54:453-72.

79. Cho HY, Jedlicka AE, Reddy SP, Kensler TW, Yamamoto M, Zhang LY, Kleeberger SR. Role of NRF2 in protection against hyperoxic lung injury in mice. Am J Respir Cell Mol Biol. 2002;26:175-82.

80. Chen XL, Kunsch C. Induction of cytoprotective genes through Nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. Curr Pharm Des. 2004;10:879-91.

81. Li N, Alam J, Venkatesan MI, Eiguren-Fernandez A, Schmitz D, Di Stefano E, Slaughter N, Killeen E, Wang X, Huang A, Wang M, Miguel AH, Cho A, Sioutas C, Nel AE. Nrf2 is a key transcription factor that regulates antioxidant defense in macrophages and epithelial cells: protecting against the proinflammatory and oxidizing effects of diesel exhaust chemicals. J Immunol. 2004;173:3467-81.

82. Barlier-Mur AM, Chailley-Heu B, Pinteur C, Henrion-Caude A, Delacourt C, Bourbon JR. Maturational factors modulate transcription factors CCAAT/enhancer-binding proteins alpha, beta, delta, and peroxisome proliferator-activated receptor-gamma in fetal rat lung epithelial cells. Am J Respir Cell Mol Biol. 2003;29:620-6.

83. Nie M, Corbett L, Knox AJ, Pang L. Differential Regulation of Chemokine Expression by Peroxisome Proliferator-activated Receptor {gamma} Agonists: interactions with glucocorticoids and {beta}2-agonists. J Biol Chem. 2005;280:2550-61.

84. Trifilieff A, Bench A, Hanley M, Bayley D, Campbell E, Whittaker P. PPAR-alpha and -gamma but not -delta agonists inhibit airway inflammation in a murine model of asthma: in vitro evidence for an NF-kappaB-independent effect. Br J Pharmacol. 2003 May;139:163-71.

85. Honda K, Marquillies P, Capron M, Dombrowicz D. Peroxisome proliferator-activated receptor gamma is expressed in airways and inhibits features of airway remodeling in a mouse asthma model. J Allergy Clin Immunol. 2004;113:882-8.

86. Woerly G, Honda K, Loyens M, Papin JP, Auwerx J, Staels B, Capron M, Dombrowicz D. Peroxisome proliferator-activated receptors alpha and gamma down-regulate allergic inflammation and eosinophil activation. J Exp Med. 2003;198:411-21.

87. Kim JE, Chen J. regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. Diabetes. 2004;53:2748-56.

88. Pernis AB, Rothman PB. JAK-STAT signaling in asthma. J Clin Invest. 2002;109:1279-83.

89. Matsukura S, Stellato C, Plitt JR, Bickel C, Miura K, Georas SN, Casolaro V, Schleimer RP. Activation of eotaxin gene transcription by NF-kappa B and STAT6 in human airway epithelial cells. J Immunol. 1999;163:6876-83.

90. Wenzel SE, Trudeau JB, Barnes S, Zhou X, Cundall M, Westcott JY, McCord K, Chu HW. TGF- beta and IL-13 synergistically increase eotaxin-1 production in human airway fibroblasts. J Immunol. 2002;169:4613-9.

91. Schedel M, Carr D, Klopp N, Woitsch B, Illig T, Stachel D, Schmid I, Fritzsch C, Weiland SK, von Mutius E, Kabesch M. A signal transducer and activator of transcription 6 haplotype influences the regulation of serum IgE levels. J Allergy Clin Immunol. 2004;114:1100-5.

92. Cazes E, Giron-Michel J, Baouz S, Doucet C, Cagnoni F, Oddera S, Korner M, Dasic G, Testi R, Azzarone B, Canonica GW. Novel anti-inflammatory effects of the inhaled corticosteroid fluticasone propionate during lung myofibroblastic differentiation. J Immunol. 2001;167:5329- 37.

93. Matsukura S, Kokubu F, Kurokawa M, Kawaguchi M, Kuga H, Ieki K, Odaka M, Suzuki S, Watanabe S, Takeuchi H, Schleimer RP, Schindler U, Adachi M. Molecular mechanisms of repression of eotaxin expression with fluticasone propionate in airway epithelial cells. Int Arch Allergy Immunol. 2004;134 Suppl 1:12-20.