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14. ABSTRACT

Beta-TrCP ubiquitin ligase receptor is required for activation of anti-apoptotic transcription factor NF-kappaB. Beta-TrCP activities are essential for v-Ras-mediated transformation of cells. As beta-TrCP proteins are pivotal to activation of the NF-kappaB pathway, up-regulation of NF-kappaB transactivation via an increase in beta-TrCP levels and activities may contribute to malignant transformation of cells. Under these conditions, an elevated expression of beta-TrCP is expected to promote cell transformation.Anti-apoptotic effect of NF-kappaB is suggested among the mechanisms implicated in NF-κB-driven transformation. NF-kappaB has been shown capable of blocking apoptosis induced by TNFalpha, ionizing radiation, or the chemotherapeutic agents. Inhibition of NF-kappaB activities dramatically potentiates apoptosis of cancer cells induced by various pro-apoptotic stimuli. These and other data indicate that NF-kappaB inhibiting agents could become useful adjuvants in anti-tumor therapies. We hypothesize that beta-TrCP activities are essential for development of breast cancer. To this end we will employ new transgenic mice with inducible dominant negative beta-TrCP2 (dn-bTrCP2) in mammary tissues in breast carcinogenesis model and determine whether inhibition of beta-TrCP function will abrogate development of breast tumors. Since beta-TrCP mediates ubiquitination and degradation of IkappaB in response to IKK-inducing stimuli, identifying the mechanisms of beta-TrCP function in mouse mammary tumors may potentially lead to design of the agents capable of inhibiting beta-TrCP function and effective for cancer prevention and therapy. The result of this study may significantly contribute to our understanding of the development of human breast tumors.

15. SUBJECT TERMS
Breast Cancer

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INTRODUCTION:

Beta-transducin repeats-containing proteins (β-TrCP) serve as the substrate recognition subunits for the $SCF^{\beta TrCP}$ E3 ubiquitin ligases. These ligases ubiquitinate specifically phosphorylated substrates and play a pivotal role in the regulation of cell division and various signal transduction pathways, which, in turn, are essential for many aspects of tumorigenesis. β-TrCP is known to mediate degradation of proteins, which exhibit anti-growth or pro-apoptotic properties (such as Emi1, Cdc25a, IκB, IFNAR1), whereas β-TrCP-mediated degradation of proteins which promote cell growth and survival (e.g., β-catenin, PRLR) is often impaired in tumors via inhibition of specific phosphorylation (reviewed in (1)). β-TrCP is required for activation of anti-apoptotic transcription factor NF-κB by activated v-Ras and v-RAF. β-TrCP activities are essential for v-Ras-mediated transformation of NIH/3T3 cells (2). As β-TrCP proteins are pivotal to activation of the NF-κB pathway, up-regulation of NF-κB transactivation via an increase in β-TrCP levels and $SCF^{\beta\text{-}TrCP}$ activities may contribute to malignant transformation of mammary epithelial cells (in addition to activation of IKK). Under these conditions, an elevated expression of β-TrCP is expected to promote cell transformation via activation of NF-κB-dependent survival pathways. Over-expression of β-TrCP2 was observed in most of the cell lines derived from human breast tumors as compared to the non-transformed cell lines, and tumor tissue samples from the patients with primary breast cancers (2). β-TrCP1/2 expression and activities are induced by oncogenes which activate MAPK pathway (including v-Ras and v-RAF) (2) and by Wnt/β-catenin signaling pathway (*3, 4*) and these pathways are often activated in breast cancers. Recent data also demonstrate that inhibition of β-TrCP activities promotes the sensitivity of breast cancer cells to anti-growth and pro-apoptotic effects of anti-cancer drugs (*5*). In addition, mammary glands of βTrcp1(-/-) female mice display a hypoplastic phenotype; conversely, the mammary epithelia of MMTV-βTrcp1 mice proliferate more and show increased NF-κB DNA binding activity and higher levels of nuclear NF-κB p65/RelA (*6*). β-TrCP plays an important role in protecting human breast cancer cells from apoptosis. Thus, we are interested to determine whether β-TrCP function is essential in mouse mammary carcinogenesis. We hypothesized that β-TrCP activities are essential for development of breast cancer. To this end we will employ new transgenic mice with inducible dominant negative β-TrCP2 (dnβ-TrCP2) in mammary tissues in a standard DMBA breast carcinogenesis model and determine whether inhibition of β-TrCP function will abrogate development of breast tumors.

BODY:

Task 1. To generate K5.rTA; TRE-dn-beta-TrCP2 double transgenic animals, we have crossed K5.rTA+/- and TRE-dn-beta-TrCP2+/+ mice. The progeny was genotyped for the presence of K5.rTA transgene, and TRE-dn-beta-TrCP2 transgenes using PCR analysis and Southern blot hybridization. Non-transgenic littermates from the same breeding were selected to use as controls in DMBA experiments. The inducibility of transgene expression was analyzed in the mammary gland of transgenic animals after treatment with Doxycycline (Figure 1).

As a result of this task, we have had sufficient number of K5.rTA; TRE-dn-beta-TrCP2 double transgenic mice for carcinogenesis experiments.

Task 2. To test whether over-expression of dominant negative beta-TrCP2 suppresses mammary carcinogenesis, we treated 56-day old virgin female K5-rTA, TRE-dnβ-TrCP2 double transgenic mice, along with non-transgenic (FVB) virgin female mice with DMBA by gavage (70 mg g^{-1} body weight) once a week for 6 weeks. To induce the expression of dominant negative beta-TrCP2 in mammary epithelium half of the animals of each genotype started receiving Doxacycline in drinking water at the time of first DMBA treatment, and continued receiving Doxacycline for the entire duration of experiment. The experimental groups will be as follows: i) double transgenic mice treated with DMBA, no Doxocycline; ii) double transgenic mice treated with DMBA, and Doxocycline; iii) non-transgenic littermates treated with DMBA, no Doxocycline; iv) non-transgenic littermates treated with DMBA, and Doxocycline. The animals were palpated twice a week for tumor appearance. Although higher than expected DMBA toxicity in mice with FVB genetic background did not allow enough time for mammary tumors to grow, we observed significant effect of transgene expression on DMBA-induced mammary hyperplasia. Histopathology revealed that Doxocycline-induced expression of dnβ-TrCP2 inhibited DMBA-induced hyperplasia in the mammary glands of K5-rTA, TRE-dnβ-TrCP2 double transgenic mice (Figure 2). The analysis of mammary glands revealed reduced nuclear accumulation of p65 in the animals with induced expression of dnβ-TrCP2 confirming that the function of βTrCP is abrogated in mammary epithelia of these mice. These data suggest that the function of βTrCP is important for early stages of mammary carcinogenesis.

The results of this task determined that inhibition of beta-TrCP function inhibits NF-κB activity and abrogates early stages of breast tumors development. K5-rTA, TRE-dnβ-TrCP2 double transgenic mice may be used as a model system to investigate the effect of β-TrCP inhibition on tumorigenesis driven by variety of oncogenes in mammary gland. The future studies will determine whether inducible expression of dnβ-TrCP2 is capable of inhibiting mammary tumorigenesis at the later stages of tumor progression.

KEY RESEARCH ACCOMPLISHMENTS:

- We have developed double transgenic mice with inducible expression of dominant negative β-TrCP2 in mammary epithelia.
- Induction of dominant negative β-TrCP2 expression in mammary epithelia inhibited activity of endogenous β-TrCP and resulted in reduced nuclear accumulation of p65/RelA member of NF-κB family of transcription factors.
- Induction of dominant negative β-TrCP2 expression in mammary epithelia attenuated DMBA-induced hyperplasia.
- K5-rTA, TRE-dnβ-TrCP2 double transgenic mice may be used as a model system to investigate the effect of β-TrCP inhibition on tumorigenesis driven by variety of oncogenes in mammary gland.

REPORTABLE OUTCOMES:

We have developed an animal model that can be used to study the function of β-TrCP ubiquitin ligase in mammary glands under variety of physiological and pathophysiological conditions (*i.e.* tumorigenesis, development, etc.).

The manuscript describing the result of this study is currently in preparation.

CONCLUSION:

The result of this study significantly contributed to our understanding of the development of human breast tumors. Anti-apoptotic effect of NF-κB is suggested among the mechanisms implicated in NF-κB-driven transformation. NF-κB has been shown capable of blocking apoptosis induced by TNFα, ionizing radiation, or the chemotherapeutic agents. Inhibition of NF-κB activities dramatically potentiates apoptosis of cancer cells induced by various pro-apoptotic stimuli. These and other data indicate that NF-κB inhibiting agents could become useful adjuvants in anti-tumor therapies. The results of this study determined that inhibition of beta-TrCP function abrogate early stages of breast tumor development. Our studies provide a rational for the development agents capable of inhibiting β-TrCP function and effective for cancer prevention and therapy.

K5-rTA, TRE-dnβ-TrCP2 double transgenic mice may be used as a model system to investigate the effect of β-TrCP inhibition on tumorigenesis driven by variety of oncogenes in mammary gland. The future studies will determine whether inducible expression of dnβ-TrCP2 is capable of inhibiting mammary tumorigenesis at the later stages of tumor progression. We are planning to cross these mice with TRE-neu mice to determine whether βTrCP activities are essential for development of breast cancer using a mouse model with inducible co-expression of Neu and dominant negative βTrCP in mammary tissues.

REFERENCES:

1. Fuchs SY, Spiegelman VS, Kumar KG. The many faces of beta-TrCP E3 ubiquitin ligases: reflections in the magic mirror of cancer. Oncogene 2004;23(11):2028-36.

2. Spiegelman VS, Tang W, Chan AM, *et al.* Induction of homologue of Slimb ubiquitin ligase receptor by mitogen signaling. J Biol Chem 2002;277(39):36624-30.

3. Noubissi FK, Elcheva I, Bhatia N, *et al.* CRD-BP mediates stabilization of betaTrCP1 and c-myc mRNA in response to beta-catenin signalling. Nature 2006;441(7095):898-901.

4. Spiegelman VS, Slaga TJ, Pagano M, Minamoto T, Ronai Z, Fuchs SY. Wnt/beta-catenin signaling induces the expression and activity of betaTrCP ubiquitin ligase receptor. Mol Cell 2000;5(5):877-82.

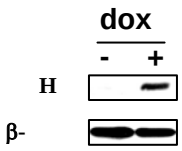
5. Tang W, Li Y, Yu D, Thomas-Tikhonenko A, Spiegelman VS, Fuchs SY. Targeting beta-transducin repeat-containing protein E3 ubiquitin ligase augments the effects of antitumor drugs on breast cancer cells. Cancer Res 2005;65(5):1904-8.
6. Kudo Y, Guardavaccaro D, Santamaria PG, *et al.* Role of F-box protein betaTrcp1 in mammary gland development and tumorigenesis. Mol Cell Biol 2004;24(18):8184-94.
7. Bhatia N, Herter JR, Slaga TJ, Fuchs SY, Spiegelman VS. Mouse homologue of HOS (mHOS) is overexpressed in skin tumors and implicated in constitutive activation of NF-kappaB. Oncogene 2002;21(10):1501-9.

APPENDICES:

Curriculum vitae is attached.

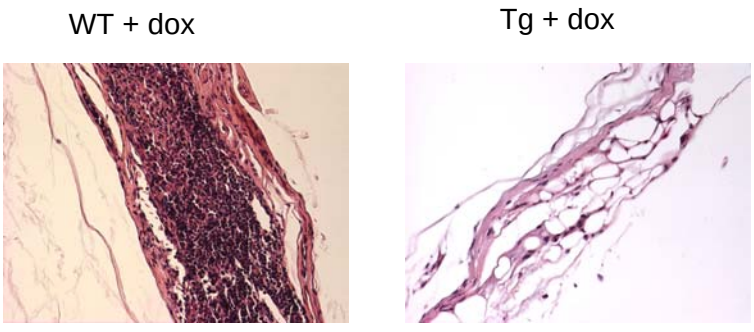
SUPPORTING DATA:

Figure 1. Induction of transgene expression in mammary gland.



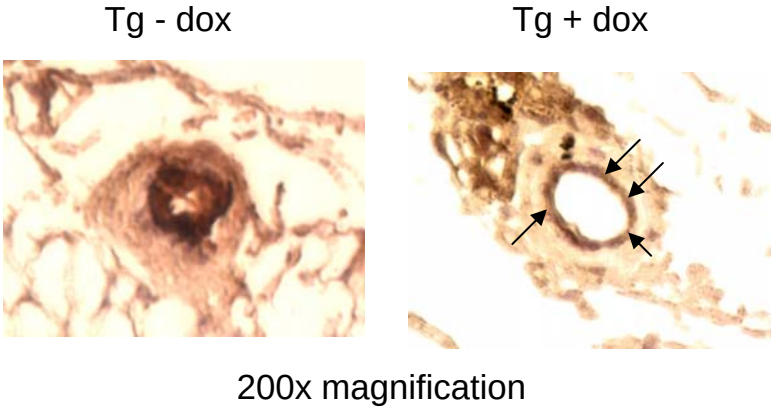
K5.rTA; TRE-HA-dn-beta-TrCP2 double transgenic animals were treated with 2 mg/ml of Doxycycline in 5% sucrose in drinking water for 7 days. dn-beta-TrCP2 expression was analyzed by immunoblot with HA-specific antibody (Santa Cruz Biotechnology).

Figure 2. Over-expression of dn-beta-TrCP2 inhibits DMBA induced mammary hyperplasia.



H&E staining of representative mammary glands.

Figure 3. Over-expression of dn-beta-TrCP2 inhibits NF-κB activity in mammary epithelia.



p65 immunostaining was performed as described previously (*7*). Arrows indicate cytoplasmic localization of p65 in the mammary epithelia of double transgenic animals expressing dn-beta-TrCP2.

CURRICULUM VITAE

June 20, 2007

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Place of Birth: Kaluga, Russia

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Education:

M.D.; 1993; Russian State Medical University, Moscow, Russia

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Postdoctoral Training

1996-1997	MD Anderson Cancer Center, Science Park Research Division, Department of Carcinogenesis, Smithville TX
1997-1998	AMC Cancer Research Center, Center for Cancer Causation and Prevention, Lakewood, CO

Awards and Honors:

1985 Secondary School #5, Kaluga, Russia. Awarded a Gold Medal as a best student.

1991 Harvard University, Cambridge MA, Travel Award for the Soviet-American Medical Student Exchange Program

1992 Travel Award of the International Medical Student Association, Aarhus, Denmark

1994 Travel Grant from the International Science Foundation. 10th International Symposium on Microsomes and Drug Oxidation", Toronto, July 18-21, 1994.

1999 Aspen Cancer Conference Young Investigator Award and Fellowship

2000 "International Skin Carcinogenesis Conference", Tuscon, AZ. Invited speaker

2002 "Society of Toxicology 41st Annual Meeting", Nashville, TN, March 17-21, 2002. Invited speaker

2002 Aspen Cancer Conference Young Investigator Award and Fellowship

2005 "International Symposium on Diet in Causation and Prevention of Cancer", Lucknow, India, March 16-19, 2005. Invited speaker.

2006 Aspen Cancer Conference Young Investigator Award and Fellowship

2007 National Cancer Center Research Institute, Tokyo, Japan. Invited speaker

2007 International Symposium on Tumor Biology, Kanazawa, Japan. Invited speaker

Lectures:

2000 The role of SCF^{HOS} ubiquitin ligase in skin carcinogenesis. In: "International Skin Carcinogenesis Conference", Tuscon, AZ, Novemer 9-11, 2000. (Invited speaker)

2001 Regulation of βTrCP ubiquitin ligase receptor. Division of Medical Oncology, Department of Medicine, University of Colorado Health Science Center, Denver, CO. October 30, 2001 (Invited speaker).

2002 Overexpression of Mouse homologue of HOS ubiquitin ligase receptor in skin tumors implicated in constitutive activation of NF-κB. In: "Society of Toxicology 41st Annual Meeting", Nashville, TN, March 17-21, 2002. (Invited speaker)

2002 Induction of HOS ubiquitin ligase receptor by mitogen signaling and its role in cell transformation. Division of Endocrinology and Program in Hormonal Cancers Research Conference, University of Colorado Health Science Center, Denver, CO. September 25, 2002 (Invited speaker).

2002 Regulation of βTrCP and HOS Ubiquitin Ligase Receptors. In International Conference: "Environmental Carcinogenesis", Moscow, Russia October 21-23, 2002 (Invited speaker).

2003 The Role of SCF^{HOS} Ubiquitin Ligase in Skin Carcinogenesis. Department of Dermatology, University of Wisconsin, Madison, WI. April 2, 2003 (Invited speaker).

2003 The Role of SCF^{HOS} Ubiquitin Ligase in Skin Carcinogenesis. Department of Dermatology, University of Michigan, Ann Arbor MI. (Invited speaker).

2005 β-TrCP Ubiquitin Ligase Receptor as a Potential Target for Cancer Prevention and Therapy. In: "International Symposium on Diet in Causation and Prevention of Cancer", Lucknow, India, March 16-19, 2005 (Invited speaker).

2005 The Regulation of β-TrCP Ubiquitin Ligase Receptor. Molecular and Cellular Pharmacology Graduate Program. University of Wisconsin, Madison, WI (Invited speaker).

2006 Mechanisms of stabilization of β-TrCP1 and c-myc mRNA in response to Wnt/β-catenin signaling. University of Texas Health Science Center at San Antonio, San Antonio Cancer Institute, SACI Seminar Series (Invited speaker).

2006 The Regulation of β-TrCP Ubiquitin Ligase Receptor. Molecular and Cellular Pharmacology Graduate Program. University of Wisconsin, Madison, WI (Invited speaker).

2007 Mechanisms of stabilization of β-TrCP1 and c-myc mRNA in response to Wnt/β-catenin signaling. International Symposium on Tumor Biology, Kanazawa, Japan. (Invited speaker)

2007 Mechanisms of stabilization of β-TrCP1 and c-myc mRNA in response to Wnt/β-catenin signaling. National Cancer Center Research Institute, Tokyo, Japan (Invited speaker).

2007 Mechanisms of stabilization of β-TrCP1 and c-myc mRNA in response to β-catenin signaling. Cancer Pharmacology Seminar, UW Paul P. Carbone Comprehensive Cancer Center, March 8, 2007 (Invited speaker).

2007 The Role of GLI2 Transcription Factor in Prostate Tumorigenesis. UW O'Brien Urology Research Center annual symposium, May 4, 2007 (Invited speaker).

Positions and Appointments:

1989-1993	Senior Lab Assistant, N.N.Blokhin Cancer Research Center, Institute of Carcinogenesis, Moscow, Russia
1990-1991	Senior Lab Assistant, Engelgardt Institute of Molecular Biology Russian Academy of Sciences, Moscow, Russia
1993-1995	Junior Scientist, N.N.Blokhin Cancer Research Center, Institute of Carcinogenesis, Moscow, Russia
1996-1997	Post-doctoral Fellow, MD Anderson Cancer Center, Science Park Research Division, Department of Carcinogenesis, Smithville TX
1997-1998	Senior Program Associate, AMC Cancer Research Center, Center for Cancer Causation and Prevention, Lakewood, CO.
1998-2004	Associate Scientist, AMC Cancer Research Center, Center for Cancer Causation and Prevention, Lakewood, CO.
2004-present	Assistant Professor, University of Wisconsin, Department of Dermatology, Madison, WI.

Grant Review Committees:

Department of Defense, Concept Awards, Breast Cancer Panel, 2006.

Department of Defense, PCTA-1, Prostate Cancer Panel, 2006.

Dutch Cancer Society

Academy of Sciences of the Czech Republic

Adhoc Reviewer for:

Molecular and Cellular Biology
The Journal of Biological Chemistry
Cancer Research
EMBO reports
International Journal of Cancer
Genomics
Cancer Letters
Molecular Carcinogenesis

Editoral Board Member:

International Journal of Biological Chemistry
International Journal of Cancer Research

Teaching Experience:

Supervised several M.S., M.D. and Ph.D. students.
Thesis advisor for Ph.D. candidate (University of Wisconsin METC program).
Serve on Thesis Committee of three Ph.D. candidates (University of Wisconsin METC program)
Supervised undergraduate students in Undergraduate Research Scholar program at the University of Wisconsin
Have supervised 5 post doctoral fellows.
Taught the genetics section of Introductory Biology - Bio 151 to undergraduate students.

Support:

Current

RO1 (Spiegelman) 2007-2012
NIH NCI
The Role of CRD-BP-regulated mRNA stability in colorectal cancers.

RO1 (Spiegelman – Co-PI) 2005-2010
NIH NCI
GR impairment in carcinogenesis: tumor suppressor role.

RO1 (Spiegelman – Co-PI) 2006-2011
NIH NCI
Stability of prolactin receptor and prolactin signaling in breast cells.

RSG-02-140-01-CNE- (Spiegelman) 2002-2007
American Cancer Society
Analysis of SCF^{HOS} ubiquitin ligase in skin carcinogenesis.

Breast Cancer Research Program 2004, (Spiegelman) 2005-2007
Concept Award
Agency: Department of Defense
The role of beta-TrCP ubiquitin ligase receptor in the development of breast cancer.

University of Wisconsin Medical School Medical Education and Research Committee; The Wisconsin Partnership Fund for a Healthy Future (Spiegelman) 2006-2007
New Investigator Program Grant
Gli2 protein stabilization in the activation of Hedgehog signaling pathway in prostate cancer.

University of Wisconsin Startup Package 2004-2007

Pending:

Prostate Cancer Research Program 2007, (Spiegelman) 2007-2010
Idea Development Award
Agency: Department of Defense
The Role of GLI2 Transcription Factor in Prostate Tumor Development

Past
ACS IRG/UCCC (Spiegelman) 2002-2003
CANCER RESEARCH SEED
Analysis of novel murine ubiquitin ligase receptor, mHOS, its role in NF-κB activation and skin carcinogenesis.

Oncology Sciences Corporation, Inc. (Spiegelman) 2002-2004

Assay for identifying specific SCF^{HOS}-ubiquitin ligase inhibitors

Cancer League of Colorado, Inc (Bhatia – fellowship) 2002-2003

Analysis of β-catenin/Tcf signaling in skin carcinogenesis.

Publications:

Peer Reviewed

1. Fuchs S.Yu., **Spiegelman V.S**., Safaev R.D., Khovanova E.M., Belitsky G.A Benzo(a)pyrene pretreatment of Drosophila simulans mutant strain results in the induction of aberrant isoform of cytochrome P-450 with increased capacity to metabolize benzo(a)pyrene. Biulleten Eksperimentalnoi Biologii i Meditsiny, 1991, 112(8):195-7.
2. **Spiegelman V.S**.*, Fuchs S.Yu., Safaev R.D., Belitsky G.A. The specificity of the genotoxic action of carcinogenic aromatic compounds on Drosophila mus mutants. Biulleten Eksperimentalnoi Biologii i Meditsiny, 1991, 112(11):521-3.
3. Fuchs S.Yu., Safaev R.D., Khovanova E.M., Ugnivenko H.G., **Spiegelman V.S**., Lytcheva T.A., Khitrovo I.A., Belitsky G.A. "A Drosophila simulans mutant strain sensitive to benzo(a)pyrene and 2-acetylaminofluorene". Mutation Res., 1992, v.268, 155-163.
4. Fuchs S.Yu., **Spiegelman V.S**., Safaev R.D., Belitsky G.A. "Xenobiotica-metabolizing enzymes and benzo(a)pyrene metabolism in the benzo(a)pyrene-sensitive mutant strain of Drosophila simulans." Mutation Res., 1992, v.269, 185-191.
5. Fuchs,S.Yu., **Spiegelman,V.S**., Belitsky,G.A."The effect of the cytochrome p-450 system inducers on the development of Drosophila melanogaster" Journal of Biochemical Toxicology, 1993, v.8, 83-88.
6. S.Yu. Fuchs, **V.S. Spiegelman**, R.I.Abdrjashitov, G.A.Belitsky. "The induction of cytochrome p-450 forms with benzo(a)pyrene in two species of Drosophila: the role in susceptibility to DDT and benzo(a)pyrene" Experim.Oncol., 1995, 17, 55-60.
7. S.Yu. Fuchs, **V.S. Spiegelman**, G.A.Belitsky. "The inducibility of various cytochrome p-450 isozymes by phenobarbital and some other xenobiotics in Drosophila melanogaster." Biochem.Pharmacol., Vol.47, No. 10, pp. 1867-1873, 1994.
8. Yakubovskaya MS. **Spiegelman V**. Luo FC. Malaev S. Salnev A. Zborovskaya I. Gasparyan A. Polotsky B. Machaladze Z. Trachtenberg AC. Belitsky GA. Ronai Z. HIGH FREQUENCY OF K-RAS

MUTATIONS IN NORMAL APPEARING LUNG TISSUES AND SPUTUM OF PATIENTS WITH LUNG CANCER. International Journal of Cancer. 63(6):810-814, 1995.
9. **V.S. Spiegelman***, S.Yu. Fuchs, G.A. Belitsky. " The expression of insecticide resistance-related cytochrome P450 forms is regulated by molting hormone in *Drosophila melanogaster*. BBRC, 232, 304-307, 1997.
10. **Spiegelman V.S**.*, Budunova I.V., Carbajal S., Slaga T.J. Resistance of transformed mouse keratinocytes to growth inhibition by glucocorticoids. Molecular Carcinigenesis, 20:1, 99-107, 1997.
11. Dobrovolskaya M.A., **Spiegelman V.S**., Belitsky G.A. Newly constructed insecticide resistant *D.melanogaster* strains with elevated sensitivity to the mutagenic effect of benzo(a)pyrene and 2-acetylaminofluorene. *Experimental Oncology*, 19 (3), 185-190, 1997.
12. Budunova I.V., Perez P., Vaden V., **Spiegelman V.S**., Slaga T.J., Jorcano J.L. Increased expression of p50-NF-κB and constitutive activation of NF-κB transcription factors during mouse skin carcinogenesis. 1999, Oncogene , 18, 7423-7431.
13. **Spiegelman V.S.,** Slaga T.J., Pagano M., Minamoto T., Ronai Z., Fuchs S.Y. Wnt/β-catenin signaling induces the expression and activity of βTrCP ubiquitin ligase receptor. 2000, Molecular Cell, 5, 877-882.
14. **Spiegelman V.S**., Stavropoulos P., Latres E., Pagano M., Ronai Z., Slaga T.J, Fuchs S.Y. Induction of β-TrCP by JNK signaling and its role in the activation of NF-κB. 2001, JBC, 276: 29, 27152-27158.
15. **Spiegelman V. S.**, W. Tang, M. Katoh, T. J. Slaga and S. Y. Fuchs. Inhibition of HOS expression and activities by Wnt pathway, Oncogene, 2002, 21, 856-860.
16. Bhatia N., Herter J. R., Slaga T. J., Fuchs S. Y., and **Spiegelman V. S.*** Mouse homologue of HOS (mHOS) is overexpressed in skin tumors and implicated in constitutive activation of NF-κB, Oncogene, 2002, 21, 1501-1509.
17. **Spiegelman V.S.,** Tang W., Chan A. M., Igarashi M., Aaronson S. A., Sassoon D. A., Katoh M., Slaga T. J. and Fuchs S. Y. Induction of Homologue of Slimb Ubiquitin Ligase Receptor by Mitogen Signaling. 2002, JBC, 277: 39, 36624-36630.
18. Tang W., Pavlish O.A., **Spiegelman V.S**., Parkhitko A.A. and Fuchs S. Y. Interaction of the Epstein-Barr virus latent membrane protein 1 with the $SCF^{\beta TrCP/HOS}$ E3 ubiquitin ligase regulates the extent of NF-κB activation. 2003, JBC, 278: 49, 48942-48949.
19. Li Y., Kumar K.G. S., Tang W., **Spiegelman V.S.**, and Fuchs S.Y. Negative regulation of prolactin receptor stability and signaling mediated by $SCF^{\beta\text{-}TrCP}$ E3 ubiquitin ligase. 2004, MCB, 24: 9, 4038-4048.
20. Tang W., Li Y., Yu D., Thomas-Tikhonenko A., **Spiegelman V.S.**, and Fuchs S.Y. Targeting β-TrCP E3 ubiquitin ligase augments the effects of anti-tumor drugs on breast cancer cells. 2005, Cancer Research, 65, 1904-1908.
21. Bhatia N., and **Spiegelman V. S.*** Activation of Wnt/β-catenin/Tcf signaling in mouse skin carcinogenesis. 2005, Molecular Carcinogenesis, 42, 213-221.
22. Li Y., Clevenger C.V., Minkovsky N., Kumar K.G.S., Raghunath P.N., Tomaszewski J.E., **Spiegelman V.S.**, and Fuchs S.Y. Stabilization of prolactin receptor in breast cancer cells. 2006, Oncogene, 25: 1896-1902.
23. Noubissi F., Elcheva I., Bhatia N., Shakoori A., Ougolkov A., Liu J., Minamoto T., Ross J., Fuchs S.Y., and **Spiegelman V.S.** * CRD-BP mediates stabilization of βTrCP1 and c-myc mRNA in response to β-catenin signaling. 2006, Nature, 441, 898-901.
24. Bhatia N., Elcheva I., Saleem M., Dlugosz A., Mukhtar H., and **Spiegelman V. S.** * Gli2 is targeted for ubiquitination and degradation by β-TrCP ubiquitin ligase receptor. 2006, JBC, 281: 28, 19320-19326.
25. Saleem M., Kweon M.H., Johnson J.J., Adhami V.M., Elcheva I., Khan N., Hafeez B.B., Bhat K.M., Sarfaraz S., Reagan-Shaw S., **Spiegelman V.S.**, Setaluri V. and Mukhtar H. S100A4 accelerates tumorigenesis and invasion of human prostate cancer through the transcriptional regulation of MMP-9. 2006, Proc Natl Acad Sci, USA, 103(40):14825-30.
26. Syed D.N., Afaq F., Kweon M.H., Hadi N., Bhatia N., **Spiegelman V.S.**, Mukhtar H. Green tea polyphenol EGCG suppresses cigarette smoke condensate-induced NF-kappaB activation in normal human bronchial epithelial cells. Oncogene. 2007, 26(5):673-82.

*- corresponding author

Invited reviews

Fuchs S.Y., **Spiegelman V.S.,** and Kumar K.G. S. The many faces of β-TrCP E3 ubiquitin ligase receptors: reflections in the magic mirror of cancer. 2004, Oncogene, 23, 2028-2036.

Fuchs S.Y., Ougolkov A. V., **Spiegelman V.S.,** and Minamoto T. Oncogenic β-Catenin Signaling Networks in Colorectal Cancer, 2005, Cell Cycle, 4, 11, 1522-39 .

Invited book chapter

Noubissi F. K. and **Spiegelman V. S.** CRD-BP/IMP-1 RNA-Binding Protein in Cancer Development. In: "RNA Binding Proteins in Development and Disease" (R. B. Denman, editor), 2007 in press

Non Peer Reviewed

1. Fuchs,S.Yu., **Spiegelman V.S.,** Safaev R.D., Belitsky G.A. Drug metabolizing enzymes in Drosophila simulans/Cytochrome P-450: Biochemistry and Biophysics", Archakov A.I., Bachmanova G.I.,eds., INCO-TNC Joint Stock Co., Moscow, 1992, P.513 - 515.
2. **V.S. Spiegelman**, S.Yu. Fuchs, G.A. Belitsky. "Molting hormone induces insecticide resistance-related forms of cytochrome p-450 in Drosophila melanogaster." In: "10th International Symposium on Microsomes and Drug Oxidation", Toronto, July 18-21, 1994, p. 389.
3. Yakubovskaya M.G., **Spiegelman V.S**., Malaev S., Belitsky G.A. and Ronai Z. "Detection of K-ras mutation in sputum of patients with lung cancer using a highly sensitive Quantitative Enriched PCR (QEPCR). " In: "PROCEEDINGS of 86th Annual Meeting of the American Association for Cancer Research", Toronto, Ontario, Canada, March 18-22, 1995, 3807.
4. Budunova I.V., Kang H., Carbajal S., **Spiegelman V.S**., Slaga T.J. Analysis of resistance of transformed mouse keratinocytes to glucocorticoids. In: "Experimental Skin Carcinogenesis Conference", UT MD Anderson Cancer Center, Science Park Research Division, October 28-31,1996.
5. **Spiegelman V.S**., Budunova I.V., Carbajal S Slaga T.J. Glucocorticoid receptor beta: possible role in the glucocorticoid resistance of transformed mouse keratinocytes. In: "6th International Congress of Cell Biology; 36th American Society for Cell Biology Annual Meeting", San Francisco, California, USA, December 7-11, 1996.
6. **Spiegelman V.S**., Budunova I.V., Carbajal S., Slaga T.J. Implication of glucocorticoid receptor splicing variant in the resistance of transformed keratinocytes to glucocorticoids. In: "PROCEEDINGS of 88th Annual Meeting of American Association for Cancer Research", San Diego, California, USA. April 12-16, 1997, 3031.
7. Budunova I.V., **Spiegelman V.S**., Carbajal S., Volk M., Slaga T.J. Alteration of NFκ-B/IκB expression during mouse skin carcinogenesis. In: "PROCEEDINGS of 88th Annual Meeting of American Association for Cancer Research", San Diego, California, USA. April 12-16, 1997, 3929.
8. Budunova I.V., **Spiegelman V.S**., Carbajal S., Slaga T.J. Analysis of resistance of transformed mouse keratinocytes to glucocorticoid. In: "PROCEEDINGS of 89th Annual Meeting of American Association for Cancer Research", New Orleans, LA, USA. March 28-April 1, 1998, 3929.
9. **Spiegelman V.S**., Budunova I.V., Slaga T.J. Possible role of alternatively spliced variants of glucocorticoid receptor in the resistance of transformed mouse keratinocytes. Keystone Symposia "Nuclear Receptor Gene Family", Incline Village, NV, USA. March 28 – April 3 1998, 257.
10. **Spiegelman V.S**., Sanko S.G., Budunova I.V., Slaga T.J. Mouse homologue of candidate-tumor suppressor $p33^{ING1}$ overexpressed during multistage skin carcinogenesis. In: "Experimental Skin Carcinogenesis Conference", UT MD Anderson Cancer Center, Science Park Research Division, November 2-5,1998.
11. Budunova I.V., Gamel D.N., Vaden V., **Spiegelman V.S**, Slaga T.J. Unbalanced expression of NFκ-B/IκB proteins and constitutive activation of NF-κB factors during mouse skin carcinogenesis. In: "Experimental

Skin Carcinogenesis Conference", UT MD Anderson Cancer Center, Science Park Research Division, November 2-5,1998.

12. **Spiegelman V.S**. and Slaga T.J. The Role of Novel Tumor Suppressor ING1 in Mouse Skin Carcinogenesis. In: "Aspen Cancer Conference", Aspen, CO, USA. July 17-21, 1999.
13. **Spiegelman V.S.**, Slaga T.J., Fuchs S.Y. The role of SCF^{HOS} ubiquitin ligase in skin carcinogenesis. In: "International Skin Carcinogenesis Conference", Tuscon, AZ, Novemer 9-11, 2000. (Invited speaker)
14. **Spiegelman V.S.**, Slaga T.J., Pagano M., Minamoto T., Ronai Z., Fuchs S.Y. The expression and activity of FBW1a ubiquitin ligase receptor is regulated by Wnt/β-catenin signaling. In: Keystone Symposia "The Molecular Basis of Cancer: Signaling to Cell Growth and Death", Taos, NM, January 9-14, 2001.
15. Fuchs S.Y., Stavropoulos P., Latres E., Pagano M., Slaga T.J, **Spiegelman V.S** Induction of β-TrCP by JNK signaling and its role in the activation of NF-κB by cellular stress. In: Keystone Symposia "The Molecular Basis of Cancer: Signaling to Cell Growth and Death", Taos, NM, January 9-14, 2001.
16. **Spiegelman V.S**., Slaga T.J., Bhatia N., Fuchs S.Y. Overexpression of Mouse homologue of HOS ubiquitin ligase receptor in skin tumors implicated in constitutive activation of NF-κB. In: "Society of Toxicology 41st Annual Meeting", Nashville, TN, March 17-21, 2002 (Invited speaker).
17. .**Spiegelman V.S**., and Fuchs S.Y Wnt signaling differentially regulates βTrCP and HOS ubiquitin ligase receptors. In: "Aspen Cancer Conference", Aspen, CO, USA. July 14-16, 2002.
18. **Spiegelman V.S**., and Fuchs S.Y, The Regulation of βTrCP and HOS Ubiquitin Ligase Receptors. In International Conference: "Environmental Carcinogenesis", Moscow, Russia October 21-23, 2002 (Invited speaker).
19. Fuchs S.Y. and **Spiegelman V.S**., Protein Ubiquitination in the Regulation of Signal Transduction. In International Conference: "Environmental Carcinogenesis", Moscow, Russia October 21-23, 2002.
20. Noubissi F., Fuchs S.Y., and **Spiegelman V.S.** Wnt/β-catenin signaling induces the expression and activity of βTrCP ubiquitin ligase receptor via the stabilization of its mRNA. In Cold Spring Harbor Meeting "Eukaryotic mRNA Processing", August 20-24, 2003.
21. Bhatia N and **Spiegelman V. S.** Activation of Wnt/β-catenin/Tcf signaling in mouse skin carcinogenesis. In: "PROCEEDINGS of 96th Annual Meeting of American Association for Cancer Research", Anaheim, CA, April 16-20, 2005, 2618.
22. **Spiegelman V.S.,** Dlugosz A., and Bhatia N. Gli2 is targeted for ubiquitination and degradation by β-TrCP ubiquitin ligase receptor. In: "PROCEEDINGS of 66th Annual Meeting of Society of Investigative Dermatology", St. Lois, MS, May 4-7, 2005, 548.
23. **Spiegelman V.S.** β-TrCP Ubiquitin Ligase Receptor as a Potential Target for Cancer Prevention and Therapy. In: "International Symposium on Diet in Causation and Prevention of Cancer", Lucknow, India, March 16-19, 2005
24. Bhatia N., Elcheva I., Saleem M., Dlugosz A., Mukhtar H., and **Spiegelman V. S.** Gli2 is targeted for ubiquitination and degradation by β-TrCP ubiquitin ligase receptor AACR Special Conference "Ubiquitin and Cancer – From Molecular Targets and Mechanisms to the Clinic", Lake Buena Vista, FL, January 18-22, 2006.
25. Clevenger C.V., Li Y., Minkovsky N., Kumar S., Puthiyaveettil R., Tomaszewski J.E., **Spiegelman V.S.**, Fuchs S.Y.. Stabilization of prolactin receptor in breast cancer cells. In: "PROCEEDINGS of 97th Annual Meeting of American Association for Cancer Research", Washington, DC, April 1-5, 2006, 3651
26. Syed D.N., Afaq F., Kweon M.-H., Hadi N., Bhatia N., **Spiegelman V.S.,** Mukhtar H.. Suppression of cigarette smoke condensate-induced NFκB activation in normal human bronchial cells by green tea polyphenol EGCG. In: "PROCEEDINGS of 97th Annual Meeting of American Association for Cancer Research", Washington, DC, April 1-5, 2006, 2256
27. Noubissi F., Elcheva I., Bhatia N., Shakoori A., Ougolkov A., Liu J., Minamoto T., Ross J., Fuchs S.Y., and **Spiegelman V.S.** CRD-BP mediates stabilization of βTrCP1 and c-myc mRNA in response to β-catenin signaling. In: Keystone Symposia "Wnt and β-catenin Signaling in Development and Disease", Snowbird, UT, April 7-12, 2006.

28. **Spiegelman V. S.**., and Bhatia N. β-catenin/Tcf signaling in mouse skin carcinogenesis In: "PROCEEDINGS of 67th Annual Meeting of Society of Investigative Dermatology", Philadelphia, PA, May 3-6, 2006, 124.
29. Noubissi F., Elcheva I., Bhatia N., Shakoori A., Ougolkov A., Liu J., Minamoto T., Ross J., Fuchs S.Y., and **Spiegelman V.S.** Stabilization of β-TrCP1 mRNA by CRD-BP in response to Wnt signaling pathway activation. In: "The FASEB Summer Research Conference on Post-transcriptional Control of Gene Expression: Mechanisms of mRNA Decay", Snowmass Village, CO, June 24-29, 2006,.
30. Noubissi F., Elcheva I., Bhatia N., Shakoori A., Ougolkov A., Liu J., Minamoto T., Ross J., Fuchs S.Y., and **Spiegelman V.S.** Mechanisms of stabilization of βTrCP1 and c-myc mRNA in response to Wnt/β-catenin signaling. In: "Aspen Cancer Conference", Aspen, CO, July 16-19, 2006.
31. **Spiegelman V.S.** Mechanisms of stabilization of βTrCP1 and c-myc mRNA in response to Wnt/β-catenin signaling. In: "International Symposium on Tumor Biology", Kanazawa, Japan. January 25, 2007