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ESPS Peer-review Report
Name of Journal: World Journal of Experimental Medicine
ESPS Manuscript NO: 3683
Title: EGCG suppresses TGF-bete signaling by interacting with TGF-beta type II receptor
Reviewer code: 00503623
Science editor: Gou, Su-Xin
Date sent for review: 2013-05-14 16:12
Date reviewed: 2013-05-15 22:23

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[ ] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[Y] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[ ] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[Y] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS
The technical aspect of the paper is well documented. However, in the light of multitude of paper on the mechanism of EGCG action the conclusion that EGCG acts through interference with receptor-ligand binding is somewhat disappointing. If EGCG does indeed bind to TGFRII, then this should be shown more convincingly (eg., cross-linking experiment). Also, Abstract, second and 3rd sentence requires restructuring.

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ESPS Peer-review Report
Name of Journal: World Journal of Experimental Medicine
ESPS Manuscript NO: 3683
Title: EGCG suppresses TGF-bete signaling by interacting with TGF-beta type II receptor
Reviewer code: 00036517
Science editor: Gou, Su-Xin
Date sent for review: 2013-05-14 16:12
Date reviewed: 2013-05-21 16:51

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[ ] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[Y]Rejection
[Y] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[ ] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

Major points 1. The origin of the cells which authors used in this study is not clear. I suggest that authors need to explain in more detail 2. The material and methods of this manuscript are hard to understand. I suggest that authors need to explain about their methods more clearly. Especially, I suggest that authors need to explain about the reason they used plasmid construction study in order to achieve their aim. 3. From their data, the discussion is too small. I suggest that authors need to discuss to their data, not just s t repeat the results. Minor points In material and methods, authors need to add the abbreviation after the name of the antibody.

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ESPS Peer-review Report
Name of Journal: World Journal of Experimental Medicine
ESPS Manuscript NO: 3683
Title: EGCG suppresses TGF-bete signaling by interacting with TGF-beta type II receptor
Reviewer code: 00742223
Science editor: Gou, Su-Xin
Date sent for review: 2013-05-14 16:12
Date reviewed: 2013-05-28 03:41

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[ ] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	
[Y] Grade D (Fair)		BPG Search:	[ ]Rejection
[ ] Grade E (Poor)	[ ] Grade D: rejected	[ ] Existed	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

This is a basic work that, with a simplistic methodology, shows that EGCG could bind to the TGF-beta type II receptor abolishing myofibroblast activation. In this way, the authors explain the antifibrotic effect of EGCG. EGCG is interesting because in the literature there are over 2000 studies on its effects on various diseases, particularly highlighting its antioxidants and pro-apoptotic properties. EGCG is a well-known scavenger of reactive oxygen species and it may also function as an antioxidant through modulation of transcriptional factors and enzyme activities. The use of EGCG could enhance the effect of conventional cancer therapies through additive or synergistic effects as well as through amelioration of deleterious side effects. In addition, there are over 30 studies about EGCG relationship with TGF-beta and its antifibrotic properties. EGCG significantly inhibited expressions of α-SMA and collagen type I mRNA and reduced α-SMA and collagen protein levels at several concentrations. Phytother Res. 2013 Mar 19. doi: 10.1002/ptr.4971. [Epub ahead of print]. Besides, EGCG inhibits TGF-β1-mediated EMT (epithelial mesenchymal transition) by suppressing the acetylation of Smad2 and Smad3 in human lung cancer cells. Cancer Lett. 2013 Feb 16. pii: S0304-3835(13)00130-4. doi: 10.1016/j.canlet.2013.02.018. [Epub ahead of print] It was also reported that EGCG can reduce ECM (extracellular matrix) production, the fibrotic marker CTGF and inhibit contraction of dermal fibroblasts. Furthermore, EGCG was able to suppress intracellular ROS, ERK1/2 kinase signalling and NF-κB activity. Inasmuch, administration of EGCG inhibited overexpression of TGF-beta and alpha-smooth muscle actin in pancreatic stellate cells. Moreover, EGCG has a potent influence on expression of Smads (downstream transcription factor of TGF-beta). EGCG suppressed the expression of Smad3 and enhanced the expression of Smad7. Biol Pharm Bull. 2007. 30(6):1091-6. For all that is published on the subject, the original point in this work is the

analysis that is done on the cytokine receptor. While the work is simple and consistent, to give importance to this study, the authors should do additional experiments. 1) They should do a dose-response study with different concentrations of EGCG and with different concentrations of TGF-β. Otherwise, they should explain in detail the reason for the choice of the dose of 50 uM of ECGC and 2ng/ml of TGFβ. 2) Why do the authors used MRC-5 cells (human fetal lung fibroblasts) to analyze the activation through the expression of SMA, and Cos-7 cells (fibroblast-like cell line derived from monkey kidney tissue) to overexpress the receptor TGF and make a lysate for immunoprecipitation experiments? They should have used the same cells, or explain in detail the use of different cells for each experiment. 3) The experiment with anti- rTGF must be done in whole cells, especially considering that EGCG has a membrane receptor. How is that receptor? EGCG has a facilitated transport and also a simple transport through the cell membrane? EGCG has higher affinity for the TGF receptor than for its own receptor? These issues must be discussed. 4) Based on the literature above described, the authors should measure collagen levels after stimulation with TGF-beta before and after adding EGCG. Besides Smad 2-3, Smad 7 should also be measured. It also would be interesting to measure the fibrotic marker CTGF (CTGF may serve as a more specific target for selective intervention in processes involving connective tissue formation fibrotic disorders). 5) Authors should make the study using other human fibroblast cell lines to verify that what is described in this paper is true or if it depends on the cell type.

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ESPS Peer-review Report
Name of Journal: World Journal of Experimental Medicine
ESPS Manuscript NO: 3683
Title: EGCG suppresses TGF-bete signaling by interacting with TGF-beta type II receptor
Reviewer code: 00051227
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[ ] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[Y] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	
[ ] Grade D (Fair)		BPG Search:	[ ]Rejection
[ ] Grade E (Poor)	[ ] Grade D: rejected	[ ] Existed	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

The authors have studied the effect of EGCG on TGF-β signaling in MRC-5 cell line. They concluded that EGCG suppresses TGF-β signaling by interacting with the TGF- β type II receptor. This short paper reports novel, a potentially interesting and an important study, however there are a couple of issue that need attention. Specific comments: 1. It seems to me that EGCG decreases GADPH protein level (Fig. 1 B) . This suggest that the effect of EGCG on TGF-β signaling in MRC-5 cell line is not specific. The authors should take into consideration that EGCG affects many processes, not only oxidation processes (is an antioxidant compound, what is mentioned in the manuscript). Please comment this problem. 2. Methods are not well described, thus not allowing the duplication of the study. For instance there is no information about solvent in which EGCG or catechin were dissolved (water or organic solvent?). If organic solvent was used, the volume added to cells should be mentioned in Material and Methods. 3. The authors should explain why they used MRC-5 cell line as an experimental model. 4. Please do not use abbreviation (especially EGCG) in title.

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ESPS Peer-review Report
Name of Journal: World Journal of Experimental Medicine
ESPS Manuscript NO: 3683
Title: EGCG suppresses TGF-bete signaling by interacting with TGF-beta type II receptor
Reviewer code: 02440441
Science editor: Gou, Su-Xin
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[ ] Grade A: Priority Publishing	Google Search:	[ ] Accept
[Y] Grade B (Very good)	[Y] Grade B: minor language polishing	[ ] Existed	[ ] High priority for publication
[ ] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
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[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

The data suggested the possibility that EGCG interferes with the interaction between the TGFR-receptor and its ligand. But the experiment is incomplete, in which precipitation of TGFRII with anti-TGFRII receptor antibody only showed that EGCG interferes with the combination of anti-TGFRII receptor antibody and TGFRII rather than EGCG interferes with the interaction between the TGR receptor. And the epitope of antigen binding antibody and the site of ligand binding to receptor can be completely different. The binding of antibody and antigen can affect or can not affect the binding of receptor and ligand. In addition, there is not the control of solution to dissolve EGCG. If EGCG change the pH value or ion strength of the solution, the binding of antibody and antigen can also be interfered. Immunoprecipitation of anti-TGR-beta and TGFRII receptor and then the experiment probed with anti-TGFRII receptor antibody should be supplemented, as well as the control of solution to dissolve EGCG.