Flaws in the peer-reviewing process : a critical look at a recent paper studying the role of CCN3 in renal cell carcinoma

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Abstract A critical look at a recently published manuscript reporting the role of CCN3 in the regulation of clear cell renal cell carcinoma (RCC) biology raises several scientific concerns, and reveals flaws in the reviewing process which appear to have resulted in the dissemination of conclusions that are not supported by proper experimental procedures. In the example presented here, the observed biological effects are attributed to a high molecular weight “CCN3” protein which is detected by a single commercial antibody that was not shown in the experimental conditions used by the authors to be a valid reagent capable of stringently detecting the “canonical” CCN3 protein. Experiments establishing that inhibiting the production of high molecular weight “CCN3” protein would reverse these biological effects were not performed. The case discussed here clearly demonstrates that unreliable data can go through peer reviewing and be published. As the data can end up being cited and used as a potential reference by new investigators in the field, we believe that such data can throw roadblocks across the scientific path of inquiry and mislead investigations. We therefore raise awareness for the need of a more stringent peer reviewing process in which assurance can be had that the strength and precision of the data have been thoroughly checked by experts in the CCN field, and previous work properly referenced.

Keywords CCN3/NOV · CCN proteins · Peer-reviewing · Renal cell carcinoma

The peer review process : strengths and weaknesses

Peer-reviewing can be defined, not exclusively, as :

- 1) The evaluation of scientific, academic, or professional work by others working in the same field (oxforddictionaries.com).
- 2) The evaluation of the performance, or the quality of work, of a member of a peer group by the experts drawn from that group. In the science research community, for example, colleagues assess the value of one’s contribution to the field by determining if the colleague’s research report is publishable in the group’s journal (businessdictionary.com)

Readers interested in a more thorough discussion of peer review might want to consult the subject published online under Analytic Quality Glossary (<http://www.qualityresearchinternational.com/glossary/peerreview.htm>)

Performed by specialists in the field of interest, peer reviewing in science, is necessary to assess the quality of experimental procedures and the relevance of the results that are reported by the authors of a manuscript. In their analysis, reviewers must also ensure that the results presented in the manuscript, are original, and that previously published work on the same topic is properly quoted. This is a matter of scientific integrity and it is a current concern in the research community.

Various aspects of reviewing, including pros and cons, have been the matter of extensive discussions in the literature and of many public debates. Recently, the preliminary findings of an extensive survey of peer reviewing performed by Sense About Science with the financial support of Elsevier, and presented at the 2009 British Science Festival, have been released (<http://www.senseaboutscience.org/news.php/87/peer-review-survey-2009>)

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A critical look at the recent publication of a manuscript reporting the role of CCN3 in the regulation of clear cell renal cell carcinoma (RCC) biology raises several scientific concerns and reveals flaws in the reviewing process which has resulted in the dissemination of conclusions that are not supported by proper experimental procedures. A more critical analysis of the paper has raised the specter of dysfunctional reviewing. It therefore behooves us as teachers and mentors to point out this type of dysfunctional reviewing and to raise red flags for young investigators who might not be acquainted with the field.

Scientific context of the CCN3 publication

Renal Cell Carcinomas (RCCs) represent 85–90 % of all kidney tumors; their incidence is on a rising trend. Frequently, the patients show metastasis at the time of diagnosis or develop distant metastases following removal of the primary tumor [Mulders et al. 1997; Sandberg and Meloni-Ehrig 1997; Walker 1998]. Because of highly anaplastic features occurring during tumor progression, and because the differences between the histologic types are sometimes ambiguous, it may be difficult to accurately categorize RCCs [Storkel et al. 1997].

According to the Heidelberg classification [Kovacs et al. 1997] that is based on genomic alterations, two major groups of RCCs were defined among the malignant neoplasms: (1) the conventional clear cell carcinomas that are characterized by the loss of the short arm of chromosome 3, the duplication of the 5q22 region and the deletion of chromosome arms 6q, 8p, 9p, and 14q (about 85 % of cases); and (2) the papillary carcinomas characterized by trisomy of chromosomes 3q, 7, 8, 12, 16, 17, and 20 and loss of the Y chromosome (10–15 % of cases).

The need for molecular markers led to assessing the usefulness of CCN3 in diagnostic and prognosis of RCCs.

As a first step in these studies, the level of CCN3 expression was evaluated in a series of xenografts established from conventional and papillary tumors (Glukhova et al. 2001). The results obtained in these early studies indicated that all of the fast growing tumors examined produced an increased level of CCN3 mRNA and increased amounts of the secreted CCN3 protein.

Along the same line, RT PCR analysis of CCN3 RNA expressed in a panel of various RCCs (Niu et al. 2005) established that CCN3 expression was associated with carcinogenesis and the progression of RCC, with the CCN3 expression level being higher in papillary than in clear cell RCCs ($p=0.040$) and higher in G1 than G2 and 3 tumors ($p=0.01$).

These observations pointed out the dual biological properties of CCN3 (Perbal 2008). CCN3 is a founding member of the CCN family of genes that encode proteins which

control fundamental biological processes governing cell proliferation, differentiation, and death from early embryonic to late stages of development (Perbal 2001, 2004, 2006; Brigstock 2003; Rachfal and Brigstock 2005; Leask and Abraham 2006; Holbourn et al. 2008; Jun and Lau 2011). Disruption of extracellular matrix-associated, and intracellular cytoplasmic and nuclear CCN3 production and/or addressing, has been associated with many different types of pathologies including cancers, fibrosis, inflammatory diseases, defective osteogenesis and stem cell fate.

In most of the cases that were studied, CCN3 showed antiproliferative activities, which are in apparent contradiction with elevated levels of CCN3 being detected in some types of tumors. As previously discussed the reduction of cell proliferation that is induced by CCN3 is probably required for the transition to the metastatic state. Some of the best documented examples are osteosarcomas and Ewing tumors in which the production of cell growth inhibitory CCN3 signifies the switch from the non-metastatic to the metastatic state, and can be used as a predictive marker of metastatic potential (Benini et al. 2005, Perbal et al. 2008b).

Scientific critique

In their attempt to assess the role of CCN3 in RCCs Liu et al. (2012), used immunochemistry to study the expression of endogenous CCN3 in a set of RCC tissues isolated from 32 patients and compared the levels of CCN3 expression with matched normal renal tissues.

Not surprisingly, as expected from previously published data (Glukhova et al. 2001; Niu et al. 2005) levels of CCN3 expression varied quite significantly between samples. Cells from the commercial human RCC line 786-O were then transfected with a CCN3-expressing vector in order to isolate transfectants that express the CCN3 protein.

According to the authors, the one stable transfectant, which was selected for further studies, was found to express high levels of intracellular recombinant CCN3. The procedure followed by the authors to study CCN3 expression in the stably transfected cells is quite straightforward, and is based on the measurement of CCN3 protein in cellular extracts obtained after extraction with RIPA buffer. This strategy is not the most ideal way to detect CCN3 produced by transfected cells as it is well-established that the bulk of CCN3 is, as are other CCN proteins, secreted in the cell culture medium where it can be easily identified (Kyurkchiev et al. 2004). Our previous studies indeed established that newly-synthesized CCN3 is rapidly secreted out of the cells (Chevalier et al. 1998).

Intriguingly, the results presented in Figure 2 of Liu et al. (2012) show that the extracts contained a single “so-called CCN3” protein with an apparent molecular weight (MW) of

73 kD. Any reader acquainted with the large bulk of published data reporting CCN3 production after transfection of either normal or tumor cells would be alerted by the fact that 73 kD is NOT the canonical apparent MW of CCN3. Intracellular CCN3 has been reported by many different groups to migrate as a 44–48 kD polypeptide whereas secreted CCN3, which is post translationally modified, shows a apparent MW of 54 kD.

In the course of our extensive studies, we and others have indeed identified CCN3 variants with different apparent MW. A higher CCN3-related protein with an apparent MW of 75 kD was indeed detected in the case of patients with CML (S. Irvine et al. unpublished results). However, we have clearly established that this high molecular weight protein was detected ONLY when the canonical 48 kD CCN3 protein was produced by the transfected cells, and we proposed that the 75 kD form corresponded to post-translational modifications of protein complexes.

With these data in mind, one could easily conclude that the biological effects observed by Liu et al. (2012) resulted either from the expression of a 73 kD CCN3 protein, or from a post transfection event that is unrelated to CCN3 expression. In the first case, careful reviewers should be definitely requesting proof of evidence, including, i) reversal of the biological effects upon inhibition of the 73 kD protein production and, ii) demonstration that the 73 kD band indeed corresponds to a CCN3-related product. In the latter case, the minimum that is required is to use a different antibody, or by blocking the ability of the antibody to recognize the 73 kD band by preincubation of the antibody with recombinant CCN3.

Furthermore, using only one transfectant to perform the proposed study is not optimal. Indeed, suggested scientific practice is to study at least 3–5 independent clones, studied and the results obtained should be averaged if possible. Moreover, from the provided materials and methods section, it is not even clear if the authors maintained selective pressure when growing their stable transfectant. One knows that in some cases, especially when the ectopically expressed protein alters growth properties of the cells, it is necessary to ensure that transfected cells still express the recombinant protein.

From the analysis of the growth rate and the adhesive and migratory properties of these transfected cells the authors conclude that CCN3/NOV exhibits growth inhibitory properties, increased cell adhesion to a selection of extracellular matrix proteins, and increased cell migration and invasion through matrigel. From the RCC standpoint, the authors report variable immunocytological detection of intracellular CCN3 protein in the various tumour samples. Papillary tumours stained positively, in agreement with previously published data (Glukhova et al. 2001; Niu et al. 2005) showing that fast growing papillary tumours secreted higher levels of CCN3/NOV than conventional and undifferentiated tumour cells.

However, the effects of CCN3/NOV on cell proliferation, adhesion, migration and invasion have been extensively documented with a wide variety of systems, including in vivo models (Perbal 2003, 2004; Holbourn et al. 2008; Jun and Lau 2011). Hence, the results reported in this manuscript bring, at best, minimal new insights regarding the biological properties of the CCN3 protein.

Consequences of a leaky peer review

The responsibility of the reviewers and of the editor is that the results published in a manuscript meet the strict requirements of quality and originality. Checking that the proper citation referencing is used in the manuscript is also part of the reviewers' mandate. In the example described above, it would appear that the reviewers and the editor let a manuscript pass in which the major conclusions—effects of CCN3 on the biological properties of cells—were flawed by a lack of critical analysis of the results and the use of a single type of antibody that was not shown, in the hands of the authors to be a valid reagent to stringently detect CCN3 protein as should have been validated using control cells or a bonafide CCN3-positive extract.

With respect to the non canonical size of the CCN3 protein, to which the authors attribute the effects on transfected cells, reviewers should have requested several controls. Establishing that CCN3 is indeed responsible for the biological effects observed, requires experiments showing inhibition of the CCN3 production in transfected cells would induce a reversal of the effects. The authors could have used specific siRNAs, inducible vectors, or site specific mutation, for example. Also, it would have been interesting to determine the effects of purified recombinant CCN3 protein on these cells.

One would also expect scientists providing reagents for the study to check the results obtained before accepting to have their name appear in the acknowledgment section of a questionable manuscript. This is perhaps more difficult to monitor, nevertheless, an important component for validating the integrity of reagents. In the present case, the authors also failed to cite properly the origin of the parental pCMV clone (Chevalier et al. 1998) that was provided for their work. This speaks to giving the appropriate credit.

Perspective

It is not a good policy to let unsubstantiated observations taint the already huge base of data that is rapidly amassing and already difficult to navigate. Thus, critical examination of manuscripts by experts in the appropriate field is necessary. Often reviewers complain that they do not have sufficient time

to review the manuscripts in detail. One can appreciate the time commitment needed. On the other hand, for the review process to work, it is necessary for the reviewers to ensure that the conclusions drawn by the authors are sustained by properly designed experiments. Such faulty reviewing may discredit the huge body of work of high quality assessments performed by the majority of reviewers who carefully analyze submitted manuscripts.

Unfortunately, there are journals where the quality of the science is not the major criterion for acceptance. Obviously, the plethora of journals and the pressure to publish, often and quickly, creates conditions such as these. It has been suggested that double-blinded peer reviewing be performed (see discussions under <http://www.qualityresearchinternational.com/glossary/peerreview.htm>), in response to a vocal group of scientists who are convinced that knowing the name of the laboratory submitting the work greatly influences the result of the peer reviewing.

The case presented here is one example that shows unreliable data can go through peer reviewing and be published, and end up being cited and used as a potential reference by new investigators in the field. Although in reality spurious data are likely to eventually be sidelined, such data can throw roadblocks across the scientific path of inquiry and mislead investigations. Thus it is our duty as teachers, and mentors to ensure that such manuscripts do not get the right of citation until the results contained are duly experimentally verified.

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References

- Benini S, Perbal B, Zambelli D, Colombo MP, Manara MC, Serra M, Parenza M, Martinez V, Picci P, Scotlandi K (2005) In: Ewing's sarcoma CCN3(NOV) inhibits proliferation while promoting migration and invasion of the same cell type. *Oncogene* 24(27):4349–4361
- Brigstock DR (2003) The CCN family: a new stimulus package. *J Endocrinol* 178(2):169–175
- Chevalier G, Yeger H, Martinier C, Laurent M, Alami J, Schofield PN, Perbal B (1998) novH: differential expression in developing kidney and Wilms' tumors. *Am J Pathol* 152(6):1563–1575
- Glukhova L, Angevin E, Lavialle C, Cadot B, Terrier-Lacombe MJ, Perbal B, Bernheim A, Goguel AF (2001) Patterns of specific genomic alterations associated with poor prognosis in high-grade renal cell carcinomas. *Cancer Genet Cytogenet* 130(2):105–110
- Holbourn KP, Acharya KR, Perbal B (2008) The CCN family of proteins: structure-function relationships. *Trends Biochem Sci* 33(10):461–473
- Jun JI, Lau LF (2011) Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets. *Nat Rev Drug Discov* 10(12):945–963
- Kovacs G, Akhtar M, Beckwith BJ, Bugert P, Cooper CS, Delahunt B, Eble JN, Fleming S, Ljungberg B, Medeiros LJ, Moch H, Reuter VE, Ritz E, Roos G, Schmidt D, Srigley JR, Störkel S, Van den Berg E, Zbar B (1997) The Heidelberg classification of renal cell tumors. *J Pathol* 183:131–133
- Kyurkchiev S, Yeger H, Bleau AM, Perbal B (2004) Potential cellular conformations of the CCN3(NOV) protein. *Cell Commun Signal* 2(1):9
- Leask A, Abraham DJ (2006) All in the CCN family: essential matrix-cellular signaling modulators emerge from the bunker. *J Cell Sci* 119(Pt 23):4803–4810
- Liu S, Liu Z, Bi D, Yuan X, Liu X, Ding S, Lu J, Niu Z (2012) CCN3 (NOV) regulates proliferation, adhesion, migration and invasion in clear cell renal cell carcinoma. *Oncol Lett* 3(5):1099–1104
- Mulders P, Figlin R, de Kernion JB, Wiltrodt R, Linehan M, Parkinson D, de Wolf W, Belldegrun A (1997) Renal cell carcinoma: recent progress and future directions. *Cancer Res* 57:5189–5195
- Niu Z, Ito M, Awakura Y, Takahashi T, Nakamura E, Ito N, Ogawa O (2005) The expression of NOV and WT1 in renal cell carcinoma: a quantitative reverse transcriptase-polymerase chain reaction analysis. *J Urol* 174(4 Pt 1):1460–1462
- Perbal B (2001) NOV (nephroblastoma overexpressed) and the CCN family of genes: structural and functional issues. *Mol Pathol* 54(2):57–79
- Perbal B (2003) The CCN3 (NOV) cell growth regulator: a new tool for molecular medicine. *Expert Rev Mol Diagn* 3(5):597–604
- Perbal B (2004) CCN proteins: multifunctional signalling regulators. *Lancet* 363(9402):62–64. doi:10.1016/S0140-6736(03)15172-0 [PubMed] [Cross Ref]
- Perbal B (2006) New insight into CCN3 interactions—nuclear CCN3: fact or fantasy? *Cell Commun Signal* 4:6
- Perbal B (2008) CCN3: Doctor Jekyll and Mister Hyde. *J Cell Commun Signal* 2(1–2):3–7
- Perbal B, Zuntini M, Zambelli D, Serra M, Sciandra M, Cantiani L, Lucarelli E, Picci P, Scotlandi K (2008) Prognostic value of CCN3 in osteosarcoma. *Clin Cancer Res* 14(3):701–709
- Rachfal AW, Brigstock DR (2005) Structural and functional properties of CCN proteins. *Vitam Horm* 70:69–103
- Sandberg AA, Meloni-Ehrig AM (1997) Renal cancer: genetic and cytogenetic aspects. In: Bertino JR (ed) *Encyclopedia of cancer*, vol III. Academic, San Diego, pp 1522–1540
- Storkel S, Eble JN, Adlakha K, Amin M, Blute ML, Bostwick DG, Darson M, Delahunt B, Iczkowski K (1997) Classification of renal cell carcinoma: Workgroup No1. Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). *Cancer* 80:987
- Walker C (1998) Molecular genetics of renal carcinogenesis. *Toxicol Pathol* 26:113–120