Reviewer’s report

Title: Increased IR-A/IR-B Ratio in Non-small Cell Lung Cancers Associates with Lower Epithelial-Mesenchymal Transition Signature and Longer Survival in Squamous Cell Lung Carcinoma

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Reviewer: Christèle Desbois-Mouthon

Reviewer’s report:

This article by Jiang and colleagues examines the expression status of the two isoforms of the insulin receptor in lung cancer. To this aim, different populations of cDNA have been analyzed: a large RNA-seq database from The Cancer Genome Atlas (TCGA) including 614 NSCLC samples and 92 adjacent normal lung tissues, a panel from OriGene Technologies containing 50 normal lung tissues, 84 adenocarcinomas and 60 squamous-cell carcinomas and a second panel containing 24 NSCLC tumors and adjacent tissues collected at the Shanghai Chest Hospital.

From bioinformatic and quantitative PCR analyses, the authors consistently observed significant decreased IR-B expression in lung tumors in comparison with normal tissue. As concerns IR-A, its expression was found to be significantly increased in tumours from the RNA-seq database while it was unchanged or even decreased in the two independent panels. As a consequence, the IR-A/IR-B expression ratio was increased in lung tumors compared to normal tissue. Samples from the RNA-seq database with a high IR-A/IR-B ratio present with a transcriptomic signature depleted in EMT genes and enriched with genes involved in oxidative phosphorylation and are associated with a better clinical outcome. Increased IR-A/IR-B ratio is also reported in tumors from different tissue localization.

There are more and more studies showing that dysregulation of insulin pathways contribute to carcinogenesis. The status of IR isoforms has not been yet evaluated in tumors from patients with lung cancer. In this respect, the present study is of obvious interest. The experimental design seems to be well performed and the paper is well presented and written.

Major point

The discrepancy between the results obtained from the RNA-seq database and those obtained from the two independent panels regarding IR-A expression raises important questions. It has been well characterized that IR-A and IR-B are produced from the alternate splicing of a unique IR pre-mRNA and that the balance between the two isoforms may be altered during pathogenesis including cancer. Up to now, increased IR-A/IR-B ratio has been reported in cancer cell and tissues as a result of IR-A overexpression associated to IR-B downregulation, thus reflecting alterations in IR splicing.
Therefore it is rather surprising that IR-A levels are not increased in tumors from the two panels. The authors attempt to justify this point by differences between sample size and content of the tumor samples. If this is true, this casts doubt on the veracity of IR-A/IR-B values and on the correlations performed from the RNA-seq database.

Other points
1. What is the status of IR-A in tumor samples from other tissues?
2. In the discussion, the authors argue that it is not clear how the differences in expression levels of IR-A and IR-B are regulated in cancer. To address this point, they evaluated promoter methylation and found no variation. This is not surprising since alterations in the regulation of IR alternative splicing have been described in other tumors such as liver tumors (Chettouh, Cancer Res, 2013).
3. How higher IR-A/IR-B ratio may push cells to depend more on IGF-II signaling through IR-A since IR-A tends to be downregulated in tumor panels (discussion section)?
4. Reference 15 is incorrect in the discussion.
5. Too much abbreviations are used in Figure 6 and Table 1.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.