Reviewer's report

Title: Characterization of genetic rearrangements in esophageal squamous carcinoma cell lines by a combination of M-FISH and array-CGH: Further confirmation of some split genomic regions in primary tumors.

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Reviewer: Susanne Gollin

Reviewer's report:

This is a very interesting, but incomplete manuscript that describes a labor-intensive study to characterize molecular karyotypes, chromosomal breakpoints, and copy number alterations in esophageal squamous cell carcinoma cell lines and confirm the presence of specific breakpoints in primary ESCC from previously untreated patients.

This manuscript requires multiple revisions before it is appropriate for publication. Constructive criticisms follow.

Major Compulsory Revisions

1. The authors report a tremendous amount of data, but do not systematically describe or discuss many of these data. Thus, this manuscript is incomplete. It could be divided into translocations, breakpoints, and copy number alterations, and each presented clearly and in detail and then discussed in light of the literature on ESCC and then other carcinomas, including lung, head & neck, breast, prostate, bladder, pancreatic, etc. How do these data compare to those available from the International Genome Project or available under NCBI or The Wellcome Trust Sanger Institute Cosmic or Conan sites on the internet?

2. For example, the authors report many different breakpoints in ESCC, but it is not clear to the reader their implications as delineated below...

2a) It is not clear how they confirmed these breakpoints (the Supplementary Table 5 said to include the BACs/PACs used appears to be missing, not listed in the additional files, and there is a different Supplementary Table 5, and the BACs or PACs used in the FISH figure (Figure 3) are not mentioned in the Figure legends).

2b) Further, it is not clear whether the tumors analyzed by FISH in Figure 3, labeled Tumor 1, for example, are all from the same tumor 1 or from different tumors from different patients. Figure 3 legend and other locations in the manuscript mention amplifications, high level or otherwise, but there is no definition of what constitutes an amplification.

2c) Further, there is no detailed description of copy number gains or losses in these cell lines or tumors in the entire manuscript, only breakpoints. Breakpoints
are interesting, but the effects of the breakpoints, gains, losses, and amplifications are usually what are considered to be important in solid tumorigenesis, not the breakpoints themselves, as in hematologic malignancies. Thus, the authors must discuss the effects of the breakpoints, that is, the gains, losses and amplifications adjacent to the breakpoints. Thus, these must be reported in the text and discussed, and should be known from the array CGH studies. They are listed cryptically in Table 2 as G/L, but no amplifications are shown, shown as lines in Figure 2, but amplifications are not distinguished and neither were cell lines, so it is difficult to determine which gains and losses were associated in the same cell line, and not discussed sufficiently in the manuscript. Nor are the gains, losses and amplifications correlated with the clinical parameters like lymph node metastasis and stage. This is more likely what is important clinically, not simply breakpoints. If not, this merits discussion.

2d. The recent literature has shown the importance of translocations in carcinomas. These authors report primarily breakpoints, and also report lists of translocations between chromosomes without reporting chromosome arms or breakpoints in the results, discussion, and Table S1 (e.g., first paragraph of the results and end of second paragraph of discussion) text of the manuscript. Translocations require chromosome arms and breakpoints to be listed according to ISCN 2009. Further, they don’t discuss whether these uninterpretable translocations without breakpoints have been reported in other carcinomas in the literature or what fusion genes at the not listed breakpoints in the translocations might be important.

3. It is not clear whether data have been deposited as required in an appropriate public international database.

4. The literature review in the Introduction is woefully inadequate. The authors indicate that the products and implications of chromosomal rearrangements have been described in only a few types of epithelial cancers. Indeed, only a few types of carcinomas have been sequenced extensively to show actual fusion genes. The genomic landscape of head and neck carcinomas was published in Science by Grandis J et al. and should be noted in the manuscript. However, the literature on chromosomal rearrangements in carcinomas is quite extensive, requiring a third edition of the book, Cancer Cytogenetics by Heim and Mitelman in 2009. Thus, in 2012, to cite only a 2007 review that you interpret as saying that the number of rearranged genes in epithelial cancers is limited is terribly inadequate. This reviewer could cite authors of a variety of such papers on various carcinomas without even going to PubMed. The Heim and Mitelman is a good place to start looking for more recent (than 2007) literature reviews before going to the primary literature.

5. To say that ESCC is one of the most common malignant epithelial cancers without population statistics from the literature is not only wrong, but requires a literature citation(s). Worldwide, in China, where is it one of the most common malignant epithelial cancers? This requires clarification and literature citation.
Minor Essential Revisions

6. What constitutes a microamplification or microdeletion? How do they differ from amplifications and deletions/gains and losses or amplifications? How are these defined?

7. The language should be edited by a native English speaking scientist familiar with the literature in this field.

**Level of interest:** An article of importance in its field

**Quality of written English:** Not suitable for publication unless extensively edited

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

**Declaration of competing interests:**

I declare that I have no competing interests