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.

Version: 1 Date: 8 February 2012

Reviewer: Ruth MacKinnon

Reviewer's report:

This is a very detailed study describing characterization of six ESCC cell lines and supporting data from patients. The novel aspects of the study appear to be

a. characterization of five of these cell lines; for all 6 cell lines combining both M-FISH and array CGH data which makes more detailed karyotypes possible
b. confirmation of previously reported aberrations, particularly amplification of the 11q13 region
c. characterising the recurrent breakpoints of splitting of the 11q13.3-q13.4 region
d. splitting of the 11q13.3-q13.4 region was an independent predictor of lymph node metastasis.

MAJOR COMPULSORY REVISIONS

1) The cell line KYSE180 has been characterized by M-FISH previously as per reference 10. This is not discussed in the introduction where it would be most helpful. Characterization of KYSE450 is said to be described in reference 10 (in Results) but it is not.

2) Some background information on previously reported findings would be very helpful. Please clearly state which are the novel findings in this paper. The reader is left to go and look up multiple references to find out what is novel here.

3) More discussion on the amplification at 11q13 in ESCC and other tumors is warranted. Place your results in the context of previous studies. In particular make it clear that 11q13 amplification is well documented, and discuss what gene(s) may be the target of this.

4) I don't find the description of the FISH method is adequate to follow. Specifically, the reference for FISH [20] describes only FISH of BAC probes, the composition of the hybridization mixture is not detailed, and protocols for the M-FISH are not described either in the manuscript or reference [20]. It is not explained exactly what is meant by “hybridized twice” (how to remove the first probe for example). This is a useful in-house approach which others might want to use.

5) Detection of recurrent breakpoint regions in primary tumor tissues. The rationale for this section is difficult to understand and needs to be more clearly explained. This is really the only section where clarifying the English will improve
meaning. See below.

6) There is clearly high level amplification at a common ~800kb region but also the breakpoints cluster at 11q13 (Figure 3A). Can you discuss whether this common amplified region gives any new information which can be used to identify an amplified oncogene? Is the amplified region defined by your results smaller than previously published? Given that these are unbalanced rearrangements with variable breakpoints (not in a single gene) and that there is high level amplification can you give more discussion to the possibility that an oncogene is the target of amplification and how this fits with the other possibilities you mention? Is there a good reason to discount amplification as the oncogenic event as you seem to be suggesting?

7) The 11q13 breakpoints (Fig 3) occurred on either side of the amplified region. How does this fit with the possibility that you discuss, that the translocations dysregulate another gene? Is the clustering of breakpoints related to the mechanism of translocation (e.g. hotspot of recombination) rather than dysregulation of a specific gene in addition to amplification?

8) Reference 25 is a nice study on the relationship between OSCC and FRA11F and discussion on this aspect would be worthwhile.

9) “Fifty-seven arm breakpoints were located at common fragile sites.” And similar on the next page (“Ten of these … at the common fragile sites”) This is misleading as most of these fragile sites have not been sequenced or accurately localized. The breakpoints have been localized in the vicinity of fragile sites. The significance of this is not discussed.

10) You state that splitting of the 11q13 region is an independent predictor of metastasis. Amplification of 11q13 rather than splitting of the region could be the relevant factor here. Has an association between 11q13 amplification and lymph node metastasis been reported? Is there a significant association when there is amplification but no splitting? Put this finding in the context of other studies linking gene aberrations in ESCC (particularly 11q13 amplification) and other tumors with prognosis, to give authority to your claim. Mention it in your abstract if it is truly novel.

11) Discussion paragraph 5, 11q13 is involved in various recurrent translocations but most or all of these are balanced and the genes involved are known, which should be mentioned so as to highlight the difference with the case in ESCC.

12) Cytogenetic nomenclature is not ISCN compliant
e.g. der(1)(1pter-1?p10::9p10->9pter)

MINOR ESSENTIAL REVISIONS

1) Introduction
a. 3rd paragraph – aCGH has been introduced for more than just translocation breakpoints, e.g. copy number aberrations such as monosomy, trisomy, deletion, amplification
b. 4th paragraph array-CGH references [17-19] presumably was not done on metaphase spreads as stated.
2) DOP-PCR is not referenced
3) BAC/PAC are not defined
4) The human genome build is not mentioned. This is a must to allow comparison to the reference map.
5) I question the use of Metafer (Metasystems) for constructing and analyzing pseudo-color images (as stated) (this is a metaphase finding system).
6) Method for labelling for array CGH is not mentioned (Agilent has more than one)
7) 11q is not mentioned in the list of gains (Unbalanced breakpoints, Results section).
8) Methods: FISH – refer to “Split genes were detected…” – refer to split regions, not split genes.

DISCRETIONARY REVISIONS
1) The English is good but could benefit from minor rewording for clarity in the Section: “Detection of recurrent breakpoint regions in primary tumor tissues”. In my opinion substituting “splitting” for “split” except in the 3rd last line (“were split in 16.9%”) improves readability of this section. Change “split occurrence” to “splitting” in the 3rd line. In the rest of the manuscript substitute “splitting” for “split” as appropriate (present tense only).
2) Last page, “2) The fragments may be…” (insert “be”)
3) Last page, “3) Truncated gene products may result…” (“result” instead of “be resulted”)
4) Statistical analysis, line 2 “characteristics” (spelling)

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests