Reviewer's report

Title: Mandatory chromosomal segment balance in aneuploid tumor cells

Version: 2 Date: 4 December 2006

Reviewer: Patricia N Tonin

Reviewer's report:

General

The study relates to chromosome copy number changes (particularly chromosome loss) that occur in solid tumour samples. Two cancer cell lines which harbour chromosome 3 anomalies were used to address the question of effect of chromosome 3p21 copy number changes due to transfer by microcell mediated chromosome transfer (MMCT) techniques. The resulting hybrids were propagated in culture or in vivo assays and chromosome 3 containing genetic material, particularly 3p21 associated material, was tracked essentially using FISH techniques. Parent of origin alleles were also investigated by PCR, using polymorphic microsatellite repeat markers. The authors found two very interesting observations. The first is that there was a tendency for loss supernumery copies of 3p21 in clones (in both in vitro and in vivo assays); and secondly, that this reduction in copy number of 3p21 was not necessary in favour of the transferred chromosome (as a result of MMCT assays). These results support the hypothesis that copy number or gene dosage of 3p21 region is important in maintaining stability in cancer cell lines.

The study addresses an interesting and emerging concept regarding copy number changes that frequently occur in cancer cells. The findings are compelling. However, there is a much needed clarification required for the sequence of experimental events described in the study (see below).

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Although the overall message of supernumerary loss of 3p21 is clear, this reviewer had difficulty in following the 'experimental design' that lead to the conclusions presented in Figure 3 C, D and E, and Figure 4 C,D, and E. The nomenclature of clone columns in both figures contained both large ("C") and small ("c") C designations. Do they represent the same thing or derivatives of the initial MMCT derived clones or sequential expansions of initial clones? In other words was exactly the relationship between the the clones listed in these two figures if any to the initial MMCT clone. Perhaps a supplementary figure showing this would clarify matters? This clarification would also be facilitated by including more information about clonal expansion in the Methods and Material section as well as the legends to these figures. For example, the percentage is referring to the number of clones from the expansion which contain supernumerary 3p21 based on shading of orange (none), yellow (one), and green (two)? If so, what does a cell with a percentage number in it but without coloured shading represent?

Figure 6 makes the point that loss of genetic material from 3p and gain of 3q material is more commonly observed than gain of 3p and loss of 3q. It is important to note in somewhere in the text that Figure 6D contains findings reported at the time of publication of MMCT results. Many of the early MMCT studies were limited by availability of polymorphic genetic markers for assaying content of hybrids or were not performed comprehensively to include genetic marker analysis of both chromosome arms. By presenting the MMCT results as such favours certain genetic regions because of the focus of study and thus may be biased. Figure 6B and 6C show normal and marker chromosome content using nomenclature shown in Figures 3 and 4. When aligned next to data presented in Figures 6D and 6E it appears that there is loss of 3p material in comparison to 3q material. However, a slightly different picture would emerge if the content were presented according to genetic material retained in each clone. This should be clarified in the both the figure and figure legend.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

**Quality of written English:** Acceptable

**Statistical review:** No