Author's response to reviews

Title: Mandatory chromosomal segment balance in aneuploid tumor cells

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Version: 4 Date: 22 January 2007

Author's response to reviews: see over
We thank all reviewers for their comments. Our answers are as follows.

**Italic: reviewer’s text**

**Blue: text from the manuscript**

**Red: change in the text**

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**Reviewer 1: Patricia N Tonin**

1. “The only comment is the use of the word "addiction" in the Discussion section (Paragraph 4). I am not certain that this word best describes the tendency of cancer cells to reduce copy number of 3p (or specific regions) in cancer cells when faced with the introduction of supernumerary chromosomes. Moreover, the 'dependency' observed can be viewed differently when compared with the tendency for tolerance of 3q gain. The maintenance of low copy number 3p arm is in line with original diploid status of normal, progenitor cells. Excess of 3p is not tolerated as nicely shown in this study and borne out by reviewing copy number changes in a large number of independently ascertained tumour samples. I am not sure what other word could be used in its place, but perhaps the statement containing 'addiction' could reflect the 'tendency' for low copy number status of 3p is favoured over higher copy number status in cancers.”

We decided to change the sentence as follows (red): “As consequence, the tumor is dependent on its gene copy number balance, but for some genes the quantitative rigidity is stronger than for others. FER is an example of a “mandatory copy number region”.

2. “Background section  
Paragraph 2: Question mark should be a period for the first sentence.”

Changed.

3. “Discussion section  
Paragraph 4:  
This paragraph requires some editing and should be carefully reviewed.  
1. The word 'however' could be placed at the beginning of most sentences in this paragraph rather than at the end or clause.  
2. Review English/American spelling changes for tumour/tumor, etc  
3. The word 'tumors' should be added as follows the sentence: "We have found the transcript of the solute carrier family 38 member 3 gene (SCL38A3) was not found in 8 tumors and was greatly reduced in .....  
4. The sentence beginning with "The LUCA region is not overlapping..." could be rewritten as follows: However, the LUCA region does not overlap our "mandatory copy number region".  
5. Add 'the' to following sentence: In #3/Hone1 the region was present on M4 marker chromosome, which was never lost in the expanded.”

Changed (red): “… **However**, after *in vivo* growth, the normal chr3 and M1 were lost more frequently than the other markers (c2 and c5 represent 20% and 40% of *in vivo* tumor cells,  

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respectively, in Figure 3). … We have found the transcript of the solute carrier family 38 member 3 gene (SLC38A3) was not found in 8 tumors and was greatly reduced in 1 out of these 9 tumors. Transcription of this gene was also impaired in 5 RCC cell lines analyzed [25]. However, the LUCA region does not overlap our “mandatory copy number region”... In #3/Hone1 the region was present on M4 marker chromosome, which was never lost in the expanded clones (see figure 3).”

Reviewer 3, Charles Buys:

“On p.15 in the 4th line from bottom the plural "s" at the end of the 2nd word should be deleted”

Changed: “The genes from this region did not change their expression in the KH39 derived MCHs (our unpublished data).”

Editor:

Abstract- Please shorten the abstract to a maximum of 350 words. Please remember to also update the Abstract details on the submission page.

Done.

Background. Euploid chromosome balance is vitally important for normal development, but is profoundly changed in many tumors. Is each tumor dependent on its own structurally and numerically changed chromosome complement that has evolved during its development and progression?

We have previously shown that normal chromosome 3 transfer into the KH39 renal cell carcinoma line and into the Hone1 nasopharyngeal carcinoma line inhibited their tumorigenicity. The aim of the present study was to distinguish between a qualitative and a quantitative model of this suppression. According to the former, a damaged or deleted tumor suppressor gene would be restored by the transfer of a normal chromosome. If so, suppression would be released only when the corresponding sequences of the exogenous normal chromosome are lost or inactivated. According to the alternative quantitative model, the tumor cell would not tolerate an increased dosage of the relevant gene or segment. If so, either a normal cell derived, or, a tumor derived endogenous segment could be lost.

Methods. Fluorescence in Situ Hybridization based methods, as well as analysis of polymorphic microsatellite markers were used to follow chromosome 3 constitution changes in monochromosomal hybrids.
Results. In both tumor lines with introduced supernumerary chromosomes 3, the copy number of 3p21 or the entire 3p tended to fall back to the original level during both \textit{in vitro} and \textit{in vivo} growth. An exogenous, normal cell derived, or an endogenous, tumor derived, chromosome segment was lost with similar probability. Identification of the lost versus retained segments showed that the intolerance for increased copy number was particularly strong for 3p14-p21, and weaker for other 3p regions. Gains in copy number were, on the other hand, well tolerated in the long arm and particularly the 3q26-q27 region.

Conclusions. The inability of the cell to tolerate an experimentally imposed gain in 3p14-p21 in contrast to the well tolerated gain in 3q26-q27 is consistent with the fact that the former is often deleted in human tumors, whereas the latter is frequently amplified. The findings emphasize the importance of even minor changes in copy number in seemingly unbalanced aneuploid tumors.

Section headings - Please use sentence case for all headings and sub-headings in the manuscript (i.e. remove all unnecessary capitals). For example Abstract not ABSTRACT.

Changed

Competing interests - If there are none to declare, please simply write ‘The authors declare that they have no competing interests’.

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