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*Letter*

**Cancer: How many genes does it take?**

Correspondence re: Zimonjic et al. Derivation of Human Tumor Cells *in Vitro* without  
Widespread Genomic Instability. *Cancer Res.*, 61: 8838-8844, 2001

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1 table, 1 figure and 6 references

Robert A. Weinberg declared at the AACR 2002 meeting “the fact that one has been unable to transform normal human cells into tumor cells has left us cancer biologists in an unbearable situation over the last decades”. In his opinion, the embarrassing situation ended with the publication of their paper in *Nature* in 1999. Therein, Hahn *et al.* reported that three defined genetic events are sufficient to convert normal human cells into malignant tumor cells (1). However, after a while it turned out that the authors mistakenly introduced at least four “defined genetic elements”; instead of a large T vector and in addition to *hTERT* and *H-ras*, a vector containing the entire SV40 early region including small and large T was used (2).

It has been shown that large T antigen induces chromosomal instability and is capable of transforming human cells after prolonged culturing *in vitro* (3). Hence, P. Duesberg proposed that aneuploidy, induced by large T antigen and long-term culturing (50 population doublings were needed), finally lead to the transformation of those cells by changing the expression rate of thousands of genes (4).

As a response, W. C. Hahn tried to clone diploid cells out of those tumorigenic HA1ER cells he generated earlier (most of the cells were highly aneuploid) and presented his data in the Dec 15 2001 issue of this journal.

In this article, the Weinberg group reports that the majority of the cells in two clones (HA1ER-2 and HA1ER-3) are diploid before and after formation of tumors in nude mice. The authors present flow cytometry results, although DNA content measurements are inadequate for karyotype analysis, because structural chromosomal aberrations and aneuploidy in the near diploid range cannot be detected. Consequently, the authors hoped that 4 SKY (Spectral Karyotyping) images of perfectly diploid karyotypes of those clones,

before and after formation of tumors in nude mice, would finally convince the last critical reader that tumorigenicity does not require genomic instability.

I am somewhat puzzled, because I got those clones from W. C. Hahn in January 2001 and could not find one diploid cell in any of those after only 1 week in culture (although chromosome numbers of the majority of the cells were in the diploid range). To explain our discrepancies, W. C. Hahn suggested that “somewhere in the handling of the cells from Boston to Berkeley” (during this time I worked in the Duesberg Lab at UC-Berkeley) “that there was selection of rare variants”. Since the cells, I received, grew very well from the start, and since mFISH analysis was undertaken just 1 week after receipt, it is very unlikely that all “diploid cells” were overgrown by aneuploid ones. Moreover, as I cultured the cells under standard conditions (37°C, 5%CO<sub>2</sub>, DMEM *Gibco*, 10% FBS *Gibco*, 1% Antibiotic/Antimycotic *Gibco*), it is highly unlikely that those chromosomal aberrations were induced in our lab.

In a recent *Science* article by J. Marx (5), Weinberg is cited again suggesting that the culture conditions in the Duesberg lab might have been too harsh. Ironically, nontumorigenic human fibroblasts (BJELB cells, I also received from R. A. Weinberg), although containing SV40 early region and hTERT, remained mainly diploid under the same conditions (74% of the cells contained no chromosomal aberrations).

Contrary to Zimonjic’s report, unbalanced chromosomal rearrangements were detected in 100% of HA1ER-2 and HA1ER-3 cells and this was before tumor formation in nude mice. Since unbalanced rearrangements are known to cause a change of gene expression in many genes (6), it was not proved that four defined genetic elements, that is small T, large T, *hTERT* and *H-ras* were sufficient to transform normal human embryonic kidney cells on their own.

Moreover, it was shown again that without chromosomal aberrations and at least transient chromosomal instability (CIN), malignant transformation of human epithelial cells does not occur. This still leaves cancer biologists in the unbearable situation mentioned above...

**Table1** Chromosomal aberrations were found in 100% of HA1ER-2 and HA1ER-3 cells by mFISH analysis<sup>a</sup> (Format and headings of the original table were used for easy comparison.).

Cell clone	Without changes <sup>b</sup>	Recurrent changes <sup>c</sup>	Random changes
<b>HA1ER-2</b>	0%	44%: der(11)t(11;18)	More than 70
61% of the cells had a		33%: der(14)t(6;14)	distinct
chromosome number in		22%: der(11)t(8;11)	chromosomal
the diploid range		11% der(2)t(2;20)	aberrations in
(41-48 chromosomes)			18 cells
<b>HA1ER-3</b>	0%	89%: der(7)t(7;17)	More than 40
96% of the cells had a		32%: der(5)t(5;13;21)	distinct
chromosome number in		32%: der(11)t(11;20)	chromosomal
the diploid range		29%: der(11)t(10;11)	aberrations in
(43-47 chromosomes)		25%: der(5)t(5;11)	28 cells
		21%: del(5q)	
		18%: der(4)t(4;11)	
		18%: +20	
		14%: der(20)t(2;20)	

<sup>a</sup> Chromosomal aberrations were determined in 28 HA1ER-3 and 18 HA1ER-2 metaphases by using a mFISH system and mFISH probes from *MetaSystems*.

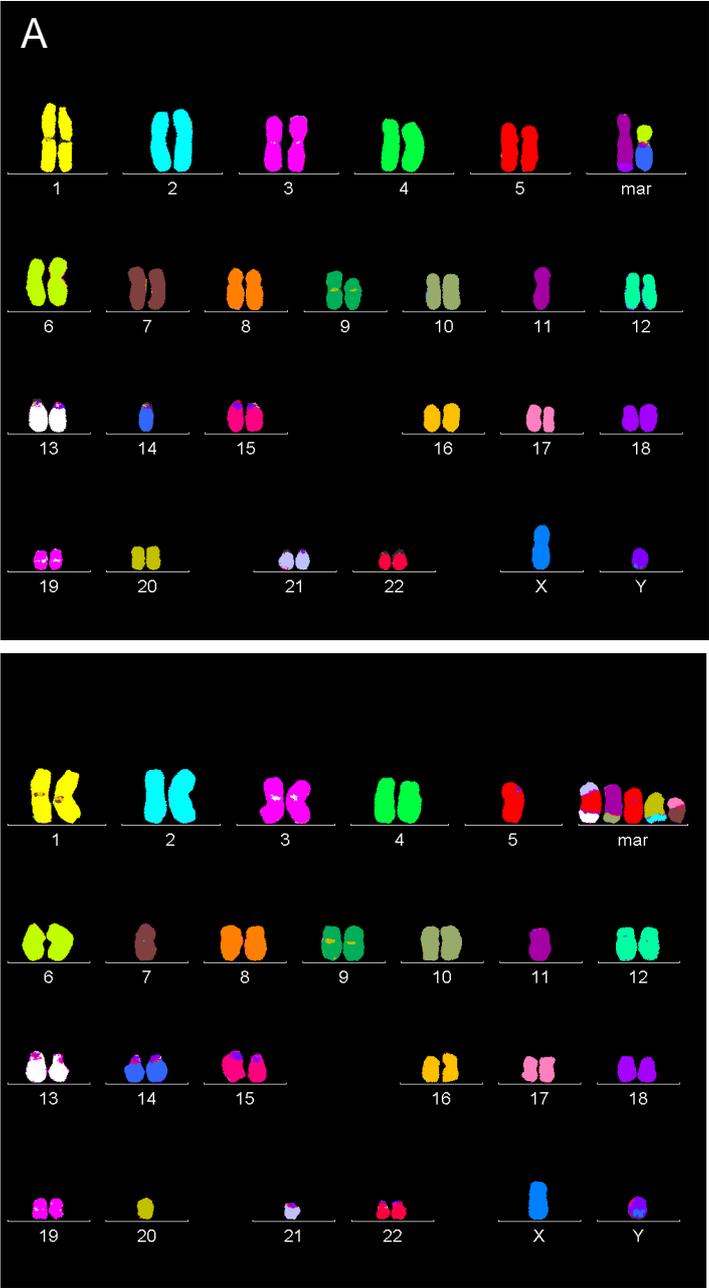
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<sup>b</sup> Without changes refers to the percentage of diploid cells lacking chromosomal aberrations.

<sup>c</sup> Frequencies of nonrandom chromosomal aberrations observed in this clone.

The abbreviations used are: der, derivative chromosome; t, translocation; del, deletion; q, long arm

**Figure 1** Two examples of pseudodiploid karyotypes of HA1ER-2 (A) and HA1ER-3 (B) cells



1. Hahn, W. C., Counter, C. M., Lundberg, A. S., Beijersbergen, R. L., Brooks, M. W., and Weinberg, R. A. Creation of human tumour cells with defined genetic elements [see comments]. *Nature*, *400*: 464-468, 1999.
2. Hahn, W. C., Dessain, S. K., Brooks, M. W., King, J. E., Elenbaas, B., Sabatini, D. M., DeCaprio, J. A., and Weinberg, R. A. Enumeration of the simian virus 40 early region elements necessary for human cell transformation. *Mol Cell Biol*, *22*: 2111-2123, 2002.
3. Ray, F. A. and Kraemer, P. M. Iterative chromosome mutation and selection as a mechanism of complete transformation of human diploid fibroblasts by SV40 T antigen. *Carcinogenesis*, *14*: 1511-1516, 1993.
4. Li, R., Sonik, A., Stindl, R., Rasnick, D., and Duesberg, P. Aneuploidy vs. gene mutation hypothesis of cancer: Recent study claims mutation but is found to support aneuploidy. *Proc Natl Acad Sci U S A*, *97*: 3236-3241, 2000.
5. Marx, J. Debate Surges Over the Origins of Genomic Defects in Cancer. *Science*, *297*: 544-546, 2002.
6. Phillips, J. L., Hayward, S. W., Wang, Y., Vasselli, J., Pavlovich, C., Padilla-Nash, H., Pezullo, J. R., Ghadimi, B. M., Grossfeld, G. D., Rivera, A., Linehan, W. M., Cunha, G. R., and Ried, T. The consequences of chromosomal aneuploidy on gene expression profiles in a cell line model for prostate carcinogenesis. *Cancer Res*, *61*: 8143-8149, 2001.