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Commentary

Spontaneous Transformation of Stem Cells *In Vitro* and the Issue of Cross-Contamination

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We have read with interest the paper "Long-term cultured human neural stem cells undergo spontaneous transformation to tumor-initiating cells", recently published by Wu *et al*. [1]. In this study the authors show spontaneous transformation of human fetal striatum neural stem cells (hsNSCs) in culture, and that the transformed cells (T1) are characterized by stem cell-like features, the expression of neural stem cell markers, abnormal karyotype and an increased growth rate. In the text they refer to previous reports on spontaneous MSC transformation [2, 3]. However, they fail to inform the readers that both these publications have later been retracted or corrected since both research groups detected that their transformed cells were cross-contaminated with cancer cells [4, 5]. In the article by Wu and colleagues, they have characterized the T1 cells by DNA fingerprinting. Most interesting, the DNA fingerprint of the transformed cells (T1) did not match the "cell of origin", and the authors explain this by genetic instability. However, we have compared the T1 fingerprint published by Wu *et al*., with public available cell line STR profiles, and find that the T1 STR profile published by Wu *et al*. is surprisingly similar to HeLa cells, Table 1. The DNA fingerprinting profile of cancer cells compared to normal cells is characterized by large differences in peak height at one or more loci, indicating genetic instability, occasional additional alleles at a locus, indicating gene duplication events, and loss of heterozygosity (LOH), at one or more loci [6, 7]. Genetic imbalance will in other words not generate a completely new fingerprinting profile.

STR profiling is currently the recommended test for cell line authentication due to its high power of discrimination and the possibility to compare the numerical code obtained from various laboratories [7]. Wu and colleagues analyzed their cells by using the PowerPlex 16 System Kit from Promega. The kit provides 15 STR markers as well as the gender determinator Amelogenin, and it has a matching probability of > 1 in 1.83×10e17 (www.promega.com). The same kit was recently used to determine the STR profile of HeLa cells [8], showing 97% identity between T1 and HeLa with only one LOH (Table 1). According to general recommendations, the profile of identical or closely related profiles should match at 80% or more of the alleles [6], and profiles with an identity level between 50 and 75% must be regarded with suspicion [7]. The HeLa profiles listed in Table 1 match T1 with 80-97% accuracy. A minor variation is seen at one locus when comparing the STR profile reported by ATCC and CLS (D13S317: 12,13.3 and 13,13.3) and Wu (D13S317: 12,14). According to the Promega Protocol for PowerPlex16, each allele at the D13S317 locus separates by 4 nucleotides, and it is therefore unclear if the allele at D13S317 13.3 is correct. There is at present several batches of HeLa cells available, and minor differences exists between them [6].

A number of scientists have pointed at the problem of cross-contamination for decades, and it is now highly recommended to authenticate cells by

DNA fingerprinting [7, 9]. Several databases for checking the fingerprinted profiles are available, such as STR profile databases at ATCC (www.lgcstandards-atcc.org) and DSMZ (www2.dsmz.de). Also a list of 360 cross-contaminated cell lines is available to help researchers quality-check their work [10], and HeLa is still the most frequent cross-contaminating cell line [10]. In conclusion, it is highly questionable if the article presented by Wu *et al*., actually describes a transforming event of hsNSCs.

Table 1: STR fingerprinting profile of hsNSC, T1 and HeLa

Cell line	D5S818		D13S317		D7S820		D16S539		CSF1PO		PentaD		D3S1358		THO1		D21S11		D18S51		Penta E		Amel.	vWA		D8S1179		TPOX		FGA		Ref.
hsNSC	10		8	9	11	13	10	14	12	13	9	10	15	17	9	9.3	29		15	17	15	18	X	16	19	10	12	11		24	25	a)
T1	11	12	12	14	8	12	9	10	9	10	8	15	15	18	4	7	27	28	16		7	17	X	16	18	12	13	8	12		21	a)
HeLa	11	12	12	14	8	12	9	10	9	10	8	15	15	18		7	27	28	16		7	17	X	16	18	12	13	8	12		21	b)
HeLa	11	12	12	13.3	8	12	9	10	9	10	NA	NA	NA	NA		7	NA	NA	NA	NA	NA	NA	X	16	18	NA	NA	8	12	NA	NA	c)
HeLa	11	12	13	13.3	8	12	9	10	9	10	8		15	18		7	27		16		7	17	X	16	18	12	13	8	12	18	21	d)

a) [1]
b) [8]
c) American Type Culture Collection (ATCC), www.lgcstandards-atcc.org
d) Cell Lines service (CLS), www.cell-lines-service.de

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