Editorial

Cross-Contamination of Cell Lines in Culture

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In a recent paper in the January 2000 issue of Laboratory Investigation (Brown et al., 2000), Dr. Brown and colleagues addressed an old problem of cross-contamination of one cell line with another (Franks and Rigby, 1975; Povey et al., 1976; Nelson-Rees and Flandermeyer, 1977; Nelson-Rees et al., 1981; O'Toole et al., 1983; Christensen et al., 1993); they have clearly demonstrated by DNA fingerprinting that the cell line ECV 304, previously considered to be a spontaneously transformed human endothelial cell line of umbilical vein origin (Takahashi et al., 1990), is actually the cell line T24/83 derived from human urinary bladder carcinoma. A supportive evidence for the identity of the ECV 304 and T24/83 cell lines was also obtained in comparative studies of the expression of G protein-coupled receptors by the ECV 304 and T24/83 cells, in studies of the ability of these receptors to initiate functional responses typical of endothelial cells, as well as in studies of serum requirements for DNA synthesis and mitogenesis of both cell lines. The paper by Dr. Brown and colleagues also addressed another issue, the necessity of re-evaluation of the results obtained previously with the cross-contaminated cells. Should the results obtained previously with the ECV 304 cells be considered as valid for human urinary bladder carcinoma epithelial cells or not? In this context, the origin and exact characteristics of the T24/83 cell line are important. Dr. Brown and colleagues gave in their paper no reference to the T24/83 cell line; the characteristics, including passage No., were not described. The authors have only mentioned that they had received the T24/83 cells from the European Collection of Animal Cell Cultures (Brown et al., 2000).

In 1973 a paper was published describing in detail the derivation of the T24 cell line from human urinary bladder transitional cell carcinoma and the characteristics of the derived T24 cells (Bubeník et al., 1973). This cell line has been made available to the American Type Culture Collection (ATCC), has served as a reference cell line for human urinary bladder carcinoma studies for more than 25 years, and is probably identical with the cell line T24/83 studied by Dr. Brown and colleagues. However, whereas the T24 cell line has been cited more than 370× in the relevant studies (ISI Philadelphia Citation Databases), it is difficult to find any reference to the T24/83 cells. Two sublines of the T24 cells were described by Flatow et al. (1987), designated as T24 A and T24 P, the T24 A being non-tumorigenic and the T24 P characterized as producing tumours in 100 % of inoculated nude mice. Another subline of the T24 cells was designated as MENNG-T24 (N-methyl-N'-nitro-N-nitrosoguanidine-transformed T24 derivative) and used for studies of malignant transformation with the carcinogen (Senger et al., 1988). The T24 cells were previously examined with regard to possible cross-contamination with other cell lines and have been found free of any cell contamination, whereas other cell lines, particularly those derived from other urinary bladder carcinomas, have frequently been found to be cross-contaminated with T24 cells (O'Toole et al., 1983; Lin et al., 1985; Christensen et al., 1993). The T24 cells were characterized with regard to their enzyme phenotype (Povey et al., 1976; O'Toole et al., 1983; Christensen et al., 1993), HLA phenotype (O'Toole et al., 1983; Christensen et al., 1993), grade of transformation (Christensen et al., 1987), differentiation (Bevers et al., 1998), and chromosomal markers (Bubeník et al., 1973; Christensen et al., 1993). No such characteristics of the T24/83 cell line have been published.

To interpret properly the findings of Dr. Brown and colleagues, detailed knowledge of the T24/83 cell characteristics is required. The obvious questions are who and when contaminated the ECV 304 cell line, are the early passages of the ECV 304 cells also contaminated (the authors have used for their investigation passages 175 and 136, respectively) and, if so, can we re-evaluate the previous findings obtained with the cross-contaminated ECV 304 cells, and conclude that these findings are valid for the well-characterized, standard T24 cell line, or for the T24/83 cells, the characteristics of which are not available. Some general rules for designation, identification, and utilization of cell lines should be respected by both, suppliers and recipients of cell lines.

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(1) The original designation of the cell line proposed by those who have established the cell line should never be changed, unless fully justified. Those who have changed the designation and developed new sublines should publish the necessary data about the sublines.

(2) Prior to utilization of a cell line supplied by the Collection of Cell Cultures, the recipients of the cell line should have the necessary information about the published characteristics of the cell line and refer to these details in their publications.

(3) Unless specifically required, as early *in vitro* generations of cell lines as possible should be utilized. The characteristics of the cell lines should be retested at regular time intervals during their further *in vitro* generations.

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