Multicentre study on standardisation of melanoma cell culture – an initiative of the German Melanoma Research Network


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Dear Sir,

Today’s basic research on cancer is not imaginable without cell culture models, which are indispensable for the definition of molecular processes, for comparison of pathophysiological phenotypes, for studying drug sensitivity or the mode of drug action etc. Malignant melanoma is the tumor with the highest number of available cell lines, which sum up to several hundreds (Herlyn, 2008), whereas the research of other tumor entities is often based on an only limited number of cell culture models. While the investigation of a larger number of cell lines increases the likelihood to better capture the biological variation representative for human diseases and to identify common mechanisms of malignancy, it bears the disadvantage that each laboratory focuses on its ‘favorite’ cells, and the comparability between the results of different laboratories is often compromised. In addition, cell culture protocols including reagents, fetal bovine serum (FBS), culture flasks, cell density, frequency of splitting etc. may have a significant influence on the phenotype. In the worst case, data generated with the same cell line in different laboratories may not be comparable.

In the recently founded German Melanoma Research Network (Melanomverbund), a consortium of 11 research groups in Germany supported by the German Cancer Aid (Deutsche Krebshilf e.V., Bonn), these problems were addressed aiming at a standardization of cell lines and cell culture conditions. At first, two standard cell lines have been defined. Here, we decided to go for A-375 and SK-Mel-28, for which the most publications are available and which are found in many cell stock collections. Thus, we tried to achieve the best possible overlap with the work of other laboratories in the melanoma field. As A-375 derived from a primary tumor and is poorly differentiated, and SK-Mel-28 is from a metastasis and shows a higher degree of differentiation (Eberle et al., 1995; Giard et al., 1973), they may be seen as representative for the high diversity of available melanoma cell lines (see Supporting Information).

In the first phase of the study, we aimed to obtain an overview on the differences in gene expression that may result from the cultivation in different laboratories. Thus, the cell lines had been sent out to the eleven groups of the research network, which cultured the cells according to the protocols established in the individual laboratories. RNA was extracted followed by reverse transcription, and the cDNAs were sent back to the study coordinators laboratory. Here, the expression of the four melanoma marker genes N-cadherin (NCAD) (Kuphal and Bosserhoff, 2006), MUC-18 (MCAM)
(Johnson, 1999), MIA (Bosserhoff, 2005) and integrin β3 (ITGB3) (Albelda et al., 1990) as well as E-cadherin (ECAD), whose expression is frequently lost in melanoma (Kuphal et al., 2004), was analyzed by quantitative real-time RT-PCR. Normal human epidermal melanocytes (NHEM) used as controls, were cultured as described previously (Massoumi et al., 2009). For normal human melanocyte cultures, we did not perform an extra study so far, as we assume that individual variations may predominate here, when cells derive from different donors. Protocols for real-time RT-PCR as well as primer sequences are given in the Supporting Information.

As depicted in Figure 1, the first experiment revealed an alarmingly high variability, ranging from no expression to 6% of the β-actin signal (N-cadherin, Muc18 and ITGB3) or from 0% to 23% (MIA). Even the melanocytic marker E-cadherin was expressed in seven samples of SK-Mel-28. The differences appeared as not due to variations of the RT-PCR as two independent amplifications revealed largely comparable results. Also different qualities of RNA extraction and cDNA synthesis appeared not as the main cause, as expression data had been normalized by the β-actin values and no clear correlation was seen between the expression levels of the different genes in the individual cDNA samples (data not shown).

Thus, the alarmingly high variation of gene expression apparently reflected a particular influence of the cell culture conditions. This variation may also partly explain some conflicting observations in the literature. The cell culture conditions were therefore tightly defined in the second phase of the study, and the same cell culture protocols were followed by all groups to reduce the variability. In this way, two new series of cDNAs were prepared. They corresponded to high density cultures (HD) and low density cultures (LD) run in parallel, to also evaluate the possible influence of the cell confluence. Again, subsequent RNA extraction and cDNA synthesis were carried out according to individual protocols, and cDNAs were sent to the study coordinator laboratories for real-time RT-PCR analysis. More details of the defined cell culture protocol are given in the legend of Figure 1 and in the Supporting Information.

With the standardized protocol, cells showed a more homogenous expression pattern, and variations decreased as particularly seen for NCAD and MIA (Figure 2). MUC-18 was now significantly expressed in both cell lines, whereas expression had been lost in several samples of the first round. NCAD was now expressed only weakly in all SK-Mel-28 samples, which had revealed significant expression in three samples of the first round, and ECAD expression was now seen only in two of the SK-Mel-28 samples. Interestingly, the differences of the mean values of expression levels were not significant when comparing experiment 1 and 2 or when comparing LD and HD cultures. However, the variability could be significantly reduced for NCAD and MIA in experiment 2, as particularly seen in the HD cultures (Table 1).
In conclusion, we aimed to determine the degree of variance that has to be considered when comparing data from different laboratories and secondly, we addressed the question whether standardized cultivation procedures may have a positive impact on reproducibility. We demonstrate that the variability can be decreased by alignment of the cell culture protocols, but it has to be noted that this was not true for all tested genes. It appears that some genes, here shown for ITGB3 and Muc-18, may be highly responsive to even small variations of the protocols that have not yet been defined. We think that this information strongly demands the usage of extra and internal controls, when correlations shall be identified with other findings. Attempts to identify the remaining critical steps in cell cultivation as well as standardization of the RNA extraction and cDNA synthesis protocols may further improve the situation. Thus, standardization of cell lines and cell culture conditions appears as inevitable, and our present work aims to initiate this discussion.

References


Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Figure S1, materials and methods, and acknowledgement.

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