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Issues to be considered when studying cancer in vitro

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Contents

Abstract

Various cancer treatment approaches have shown promising results when tested preclinically. The results of clinical trials, however, are often disappointing. While searching for the reasons responsible for their failures, the relevance of experimental and preclinical models has to be taken into account. Possible factors that should be considered, including cell modifications during in vitro cultivation, lack of both the relevant interactions and the structural context in vitro have been summarized in the present review.

Keywords: In vitro; Cell culturing; Extracellular matrix; Tumour microenvironment; Tissue stiffness

1. Introduction

Intensive efforts within cancer research resulted in a number of novel anticancer agents. Positive results of treatments with some of them have been documented in patients. The most promising examples include anti-epidermal growth factor receptor (EGFR) monoclonal antibodies in some patients with metastatic colorectal cancer [1] and metastatic breast cancer [2] or tyrosine kinase inhibitors in some patients with chronic myelogenous leukemia [3] or non-small cell lung cancer [4]. However, the efficacy of many of the newer biological and targeted therapies is confirmed in some individuals only. For example, only patients with colon tumours expressing wild-type *KRAS* or with *HER2/neu*-positive breast cancer are likely to benefit from

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the treatments with the anti-EGFR monoclonal antibodies [1]. Treatment with tyrosine kinase inhibitor (imatinib) is efficient in patients with *BCR-ABL* translocations [5].

Except of the encouraging achievements, however, negative results of clinical trials are also reported. To mention some of them, treatment with matrix metalloproteinase (MMP) inhibitors showed no survival advantage for the patients compared to conventional treatment modalities or even lead to poorer survival and had to be terminated [6–8]. Clinical trials with Fe-chelator Triapine® as anticancer drug, either alone in patients with metastatic renal cell carcinoma [9] or in combination with gemcitabine in patients with advanced pancreatic adenocarcinoma [10], were terminated as they did not give any benefit for the patients at the used doses and schedules. Clinical trials with anti-EGFR agent-combining regimes in advanced non-small cell lung cancer patients showed no survival advantages over chemotherapy alone [11]. Based on in vitro observations of a dose–response effect, high-dose chemotherapy with stem-cell transplantation has been evaluated for the treatment of various solid cancers and compared with conventional modalities. Although event-free survival was often found to be advantageous in favour of high-dose chemotherapy, no significant benefit in overall survival, apart from few exceptions, or even negative results were reported [12,13]. Current gene therapy has not proven very successful in clinical trials [14,15].

Understanding the reasons for the failures of clinical trials, in spite of promising preclinical results, is of high importance from scientific point of view. While searching for them, the relevance of experimental and preclinical models has to be considered. Predictions of treatment efficacies in clinical trials based on in vitro studies [16] and even preclinical mouse models [8] were reported to lead to disappointments in a number of cases. For example, Fe-chelator Dp44mT did not lead to Fe depletion within the tumour, despite its high activity at inhibiting Fe uptake from transferrin and inducing Fe mobilisation from cells in cultures [17]. Many of tumours that relapsed after treatment by radiotherapy appeared within irradiated regions, despite in vitro and preclinical data indicating that the doses used would kill >99% of both the tumour and endothelial cells [18]. Moreover, in a randomized trial of a regime combining application of a monoclonal antibody against EGFR, there was no correlation between EGFR expression and tumour response and/or symptom improvement in patients with non-small cell lung cancer [11]. In a phase III trial with a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), VEGF expression, assessed by in situ hybridization in primary tumour samples, did not correlate with objective response in advanced breast cancer patients [11].

Apart from inconsistent findings between in vitro studies and clinical trials, there appear also reports that document discrepancies between results obtained under in vitro and in vivo (e.g. [19–26]) or ex vivo (e.g. [25,27]) conditions. In fact, many researchers realized limitations of usually used in vitro and in vivo experimental systems already long time ago. It has been recognized that cell culture systems, and even animal carcinogenesis models, may not accurately represent the complexity and the true physiologic state of the diseased human tissue [28] and simplification inherent to vitro approaches might be achieved at the cost of physiological relevance [29].

In order to be able to correctly interpret findings of in vitro and preclinical experiments, it is crucial to understand the physiological and pathological relevance of the experimental conditions. Therefore, factors governing cell behaviour need to be precisely identified and the alterations of cells in cultures that are artifacts of culturing (e.g. cell modifications due to long-term culturing or cell processing) need to be distinguished. Unfortunately, inconsistency in experimental protocols and conditions used makes comparisons of the results among laboratories not easy. Issues that should be considered in experiments utilizing in vitro cell cultures and in interpreting their findings, especially when studying cancer, have been summarized in the present review.

2. Cell modifications during in vitro cultivation

Use of cell cultures offers considerable simplification for biologically oriented research. Nevertheless, evidence suggests that cells undergo changes when placed in cultures [30–35] (Tables 1 and 2), which gives rise to doubts whether cell lines are representatives of their original tissues [36–38].

2.1. Representativeness of tumour cell lines

Many tumour cell lines have been derived from metastases, pleural or ascitic effusions (references in [36,39]). Such cells constitute a population of cells in a late stage of tumour evolution (references in [36]), which may differ from their corresponding primary tumours with respect to ploidy [36], antigenicity and immunogenicity and therefore the response to therapies [40,41]. Cell lines developed from primary tumours were claimed to be representatives of the tumour specimen from which they were derived [36,42,43]. Nevertheless, difficulties to establish even primary cultures from primary tumours [36,39,44–46] with majority of outgrowths arising from normal cells within the specimen [45,46] have often been reported. Success rates for cell line establishments from primary and metastatic breast cancers were 10% and 25%, respectively [47]. Low success rates in the range of 1–10% were reported for the establishment of leukaemia–lymphoma cell lines. It was suggested that the original primary cells might need to possess special features that make them prone to immortalization in vitro and only samples containing such cells may evolve into cell lines [48]. It was even mentioned that only rare specimens of breast cancer develop into cell lines [33,46]. Furthermore, the growth of primary carcinoma cells was found to be considerably slower than that of carcinoma cell lines (references in [45]). This is