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Cell Cultures as Reliable Models in Experimental-Oncopharmacology

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The aim of our study was to summarize the role of various cancer cell cultures (human, rat, chicken; established from different types of cancer; monolayer cell cultures and 3D cancer cell colonies; primary cell cultures and permanent cell lines, tumor and non-tumor cells) used in our investigations for the evaluation of cytotoxic / antitumor activity of compounds (a total of 24 compounds) with different chemical structures and chemical / physicochemical characteristics – ammonium vanadate (NH_4VO_3) and as well as ionophore antibiotics (monensin), non-steroidal anti-inflammatory drugs (NSAIDs, meloxicam), cholic acids (ursodeoxycholic acid, UDCA), Mannich bases (BAMP = N,N'-bis(4-antipyrylmethyl)-piperazine; TAMEN = N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane) and their metal complexes. The advantages and disadvantages of cell cultures used as model systems in the experiments as well as strategies to meet the challenges of such *in vitro* models are presented.

Key words: cell cultures, model systems, cancer, cytotoxicity, experimental oncopharmacology

Introduction

Cell cultures have successfully served as experimental models for investigations in the fields of experimental oncology and oncopharmacology for many years and have contributed to our understanding of tumor biology and mechanisms of cancerogenesis as well as to the introduction of diagnostic, prophylactic and treatment strategies in clinical practice [6, 11, 13]. There are different types of cell cultures, including primary cell cultures (PCC) and permanent cell lines (PCL); tumor and non-tumor cell cultures; suspension, monolayer (2D) and 3D cell cultures; cell cultures established from different organisms and from different histological types of tumors, etc. Each cell culture has its unique biological properties. Knowing the advantages and disadvantages of individual cell lines will facilitate the choice of the most appropriate model systems for certain scientific purposes and will help us to improve their predictive capacity for cancer drug development / discovery. The aim of the present study was to summarize the potential role of various cell cultures used by our group for the evaluation of cytotoxic / antitumor activity of compounds with different chemical structures and chemical / physico-chemical characteristics.

Materials and Methods

The cytotoxic activity of 24 compounds was evaluated using a wide range of cancer cell lines: i) chicken - LSCC-SF-Mc29 (hepatoma), ii) rat - LSR-SF-SR (sarcoma) and iii) human – A549 (non-small cell lung cancer), MCF-7 (luminal A type breast cancer), SK-BR-2 (Her-2 positive breast cancer), Caco-2 (colorectal cancer), HeLa (carcinoma of the uterine cervix); HepG2 (liver cancer), 8MGBA (glioblastoma multiforme), A431 (squamous cell carcinoma) and its multidrug resistant clones A431-MDR1, A431-MRP1 and A431-ABCG2. Non-tumor human (embryonal Lep-3 and MRC-5) as well as bovine (MDBK - Madin Darby bovine kidney cells) cell lines were also included in the experiments. The compounds tested for cytotoxic activity as well as cell cultures used as model systems are presented in Table 1.

The cells were grown as monolayer (2D) cultures in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 5-10% fetal bovine serum and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) or as 3D cancer cell colonies in a mixture of 2X DMEM medium and 0.9% agar (1 : 1, vol. : vol.). The investigations were performed by short-term (24-72h) experiments with monolayer cell cultures and methods with different molecular targets and mechanisms of action (MTT test, neutral red uptake assay, crystal violet staining, trypan blue dye exclusion technique, double staining with acridine orange and propidium iodide) and long-term experiments (14-30 days) with 3D colony-forming method as it was earlier described [1, 4].

Results and Discussion

The cytotoxic activity of 24 compounds with different chemical structures and chemical / physicochemical properties was evaluated in our investigations using a wide range of cancer permanent cell lines (**Table 1**): human, rat, chicken; cultures established from different types of cancer; grown as 2D- or 3D- cell cultures; sensitive and multidrug resistant cancer cells. The examined compounds were found to reduce to varying degrees the viability and proliferation of the treated cells, each cell culture expressed different rate of sensitivity to the cytotoxic effect of each individual compound. Examples of hierarchical orders based on the chemosensitivity of the model cell lines are presented in **Table 2**. The results / experience obtained by us can be summarized as follows:

i) Chicken hepatoma and rat sarcoma cells

LSCC-SF-Mc29 chicken hepatoma and LSR-SF-SR rat sarcoma cells were found to be highly sensitive to the cytotoxic activity of the compounds examined. Both cell lines (LSCC-SF-Mc29 and LSR-SF-SR) are valuable model systems for the search of new anticancer agents because of at least five reasons: i) they are easily grown as 2D cell cultures and 3D cancer cell colonies; ii) implanted subcutaneously in immunocompetent chickens or rats, respectively, the cells induce tumor development and are useful for *in vivo* investigations in the fields of experimental oncopharmacology and tumor immunology; iii) these cells contain / express *v-myc* (LSCC-SF-Mc29) or *v-src* (LSR-SF-SR) oncogene. The cellular analogues of these genes are known to be involved (when their expression and/or activity is / are not properly regulated) in the pathogenesis of a wide range of cancers in humans and animals. Myc and Src genes and their products are attractive targets for the development of innovative new anticancer strategies [7, 12]; iv) the high sensitivity of chicken hepatoma and rat sarcoma cells makes them suitable models for primary screening for new anticancer agents; v) while a large number of mammalian (for example human, mouse) cell lines are known, the amount of the available avian permanent cell lines is quite limited. The relatively low

Table 1. Compounds tested and cell cultures used as model systems for the evaluation of their cytotoxic activity

	Compound(s)	Number of the compounds tested	Cell cultures used as experimental models
1.	Meloxicam (Mel) and its Zn(II), Cu(II), Co(II) and Ni(II) complexes	5	LSCC-SF-Mc29; LSR-SF-SR, HeLa, 8MGBA
2.	Monensin and its Mg(II), Ca(II), Mn(II), Co(II), Ni(II) and Zn(II) complexes	7	LSCC-SF-Mc29; LSR-SF-SR, MCF-7, HeLa, A549, HepG2, 8MGBA, A431 and its multidrug resistant clones A431-MDR1, A431-MRP1 and A431-ABCG2, Lep-3
3.	Ursodeoxycholic acid (UDCA) and its Zn(II), Cu(II) and Ni(II) complexes	4	LSCC-SF-Mc29; LSR-SF-SR, MCF-7, HeLa, A549, HepG2, Lep-3
4.	Complexes of Ni(II) with Mannich type ligands BAMP or TAMEN	6	LSCC-SF-Mc29; LSR-SF-SR, MCF-7, SK-BR-3, Caco-2, HepG2, 8MGBA
5.	Mixed ligand Cu(II) complex Cu_2BAMPdipyCl_4	1	LSCC-SF-Mc29; LSR-SF-SR, A431, A431-MDR, A431-MRP, A431-ABCG2
6.	Ammonium vanadate (NH_4VO_3)	1	LSCC-SF-Mc29; LSR-SF-SR, MCF-7, HeLa, HepG2, Lep-3, MRC-5, MDBK
Total		24 compounds	13 cell cultures – 10 tumor and 3 non-tumor

BAMP = N,N'-bis(4-antipyrylmethyl)-piperazine; TAMEN = N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane; dipy = 2,2 bipyridyl

amount of commercially available monoclonal antibodies against avian and rat antigens limits to some extent the possible applications of these cell lines in laboratory practice.

ii) Human cervical carcinoma cells

HeLa was the first human cell line established in culture [5]. HeLa cells are well studied and used for many decades all over the world as a model system to carry out a wide range of investigations [13]. It is worth to be mentioned here that two Nobel prizes for physiology or medicine have been awarded for discoveries achieved by the "help" of HeLa cells: the link between human papilloma virus (HPV) and cervical cancer (2008, Harald zur Hausen) and the role of telomeres and the enzyme telomerase in preventing the ends of chromosomes from degradation (2009, Elizabeth Blackburn, Carol Greider, and Jack Szostak). HeLa cells are particularly suitable in the search for new diagnostic, prognostic and therapeutical approaches for human cervical cancer. The results obtained by us reveal that these cells exhibit moderate sensitivity to the cytotoxic effect of the compounds investigated as compared to the other human cancer cells and are more resistant than rat sarcoma and especially chicken hepatoma cells (**Table 2**). The HeLa cells contain human papilloma virus type 18 (HPV – 18) [3, 10]. It will be interesting to evaluate comparatively the influence of one and the same agent on the viability and proliferation of human cervical carcinoma cells containing different high-risk oncogenic (e.g. HPV-16, HPV-18) and low-risk oncogenic (e.g. HPV-6, HPV-11) types HPV.

iii) Human breast cancer cell lines

MCF-7 and SK-BR-3 cell lines were established from different subtypes of human breast cancer: luminal type A (MCF-7) and HER-2 positive (SK-BR-3) breast cancer.

Table. 2. Hierarchic orders of cell lines according to their sensitivity to the cytotoxic effect of compounds examined

Compound	Method	Treatment period, h	Hierarchic order
$Ni_2B(CH_3COO)_4$	MTT	72	MCF-7 (57.2)* > HepG2 (81.8) > 8MGBA (100.8) > Caco2)172.3) > SKBR-3 (2016.1)
Ni_2BCl_4	MTT	72	MCF-7 (94) > HepG2 (149.5) > 8MGBA (221.6) > Caco-2 (238.8) > SKBR-3 (268.3)
Cu-Mel	MTT	72	LSCC-SF-Mc29 (32) > LSR-SF-SR (53) > 8MGBA (304) > HeLa (306)
Co-Mel	MTT	72	LSCC-SF-Mc29 (42) > LSR-SF-SR (45) > 8MGBA (313) > HeLa (429)
Zn-UDCA	MTT	72	LSCC-SF-Mc29 (68) > LSR-SF-SR (102) > HepG2 (143) > HeLa (149) > A549 (161) > Lep3 (>200)
Cu-UDCA	MTT	72	LSR-SF-SR (<50) > LSCC-SF-Mc29 (37) > HepG2 (159) > A549 (169) > 8MGBA (>200) = HeLa (>200) = Lep3 (>200)
NH_4VO_3	MTT	72	LSR-SF-SR (1.0) > MRC-5 (1.0) > LSCC-SF-Mc29 (1.5) > HepG2 (2.1) > HeLa (7.0) > MCF-7 (7.7) > Lep3 (8.0)
	NR	72	LSCC-SF-Mc29 (1.4) > LSR-SF-SR (2.0) > HepG2 (3.8) > HeLa (4.4) > MRC-5 (7.8) > MCF-7 (8.4)

* – Cytotoxic concentration 50 (CC_{50}, μM) that reduce the percent of viable cells by 50% as compared to the non-treated control; All hierarchic orders start with the most sensitive cell line (with the lowest CC_{50} value of the compound); MTT – thiazolyl blue tetrazolium bromide test; NR – neutral red uptake cytotoxicity assay; B = BAMP = N,N'-bis(4-antipyrylmethyl)-piperazine.

The experimental data obtained showed that SK-BR-3 cells were less sensitive to the cytotoxic activity of Ni(II) complexes with BAMP ($Ni_2(BAMP)(CH_3COO)_4$ and $Ni_2(BAMP)(Cl)_4$) as compared to MCF-7 cells (Table 2). In addition, MDA-MB-231 (triple negative breast cancer, TNBC) cell line was more resistant to the cytotoxic activity of Zn(II)/Au(I) and Zn(II)/Ag(I) complexes with Schiff bases (Salen, Salampy and Saldmen) than MCF-7 cells [14]. The observed difference in sensitivity of these cells can be explained at least partially by tumor heterogeneity phenomenon [2] that makes each tumor / tumor cell line a unique biological system. At the same time, luminal A type breast cancer has a more favorable prognosis as compared to Her-2 positive and triple negative breast cancer. Investigations performed with cell lines established from different breast cancer cell types will allow better understanding of breast cancer biology and behavior and will facilitate the identification of new treatment strategies, especially for TNBC for which currently there is no targeted / specific treatment available.

iv) Multidrug resistant cancer cells

The cell line A431 (human squamous cell carcinoma) and its clones expressing mdr1 (A431-MDR1), mrp1 (A431-MRP1) or abcg2 (A431-ABCG2) gene were also included in our studies. It was found that mixed ligand complex $Cu_2BAMPdipyCl_4$ as well as monensin and its metal complexes decrease significantly viability and proliferation of both – sensitive parental A431 cell line and resistant cell clones. Drug resistant cancer cell lines can be established by genetic manipulation; continuous culturing in the presence of gradually increasing concentrations of particular anticancer agent(s) (starting by non-toxic concentration); cultivating the primary cell culture derived from tumor tissue in medium containing high concentration of antitumor agent/s. The last

two technical approaches represent the well known situations in clinical practice where oncology patients can develop drug resistance during the prolonged course of cancer treatment (acquired drug resistance) or demonstrated drug resistance from the very beginning of chemotherapy (pre-existing, intrinsic drug resistance) [9]. Drug resistance is among the main obstacles preventing successful managing of cancer diseases. (Multi) drug resistant cancer cell lines are absolutely necessary for better understanding of this phenomenon as well as for the discovery of sensitizing agents and effective treatment strategies.

v) 2D- and 3D- cell cultures

Monolayer (2D) cell cultures were used in our investigations to evaluate the "quick" cytotoxic effect of the compounds tested (in short-term experiments lasting usually 24-72h) by MTT, NR, CV, TB and AO/PI assays. In order to examine the 'duration" of the cytotoxic effect we carried out CFM based on the natural ability of cancer cells for anchorage-independent 3D growth in semi-solid medium. These long-term experiments last 2-4 weeks and provide more adequate information about cytotoxic activity of the compounds tested. Conventional monolayer cell cultures are easier to maintain, suitable for routine culturing, well studied and allow application of wide range cytotoxicity assays. 3D-cell cultures are more "realistic" model systems and represent better tumor / tumor cell biology and behavior.

vi) Primary cultures

Permanent cell lines were proved to be suitable tools for the needs of biomedicine and biotechnology. They have many advantages as model systems in experimental oncopharmacology such as adaptation for propagation in laboratory conditions, accessibility and availability, their biological features are well characterized, etc. On the other hand, a large number of *in vitro* passages of PCL can result in significant genetic and epigenetic changes that do not exist in the tissue of origin. Even one and the same PCL cultured in different laboratories / conditions may show some different characteristics. That is why primary cell cultures are more "close" to the initial tissue. PCC can be useful in the search for new anticancer agents and for the needs of personalized medicine. From practical point of view the establishment of PCC meets some challenges such as the presence of unwanted stromal fibroblasts, possible microbial contamination (for example in the case of gastric or colorectal cancer), short life, etc.

vii) Non-tumor cell cultures

Non-tumor permanent cell lines were included for comparative purposes in our investigations. The results obtained reveal that these non-tumor cells are usually also highly sensitive to the cytotoxic activity of the compounds examined. This is not surprising because the non-tumor PCL are usually established from embryonal tissues – it is well known that tumor and embryonal cells share some common characteristics including high proliferative activity, expression of some antigens. In addition, embryonal cells are highly sensitive to the influence of the chemical agents in their microenvironment whereas cancer cells have been selected during tumor progression to become more and more aggressive and well adapted [2]. In our opinion, PCC obtained from transplantable tumors in laboratory animals and healthy cells from the same tumor-bearing animals (lymphocytes, macrophages, bone marrow cells, etc) are suitable model systems in the field of experimental oncology representing the situation of patients under cancer chemotherapy. Special attention deserve bone marrow, liver, kidney and heart cells (tissues that are frequently attacked during cancer chemotherapy), but the establishment of such PCC is challenging and can be with low success.

Conclusion

Cell cultures are suitable model systems in the search for new agents with promising antitumor properties and identification of their molecular target(s) and mechanism(s) of action. Each cell line has its own individual features and advantages providing specific benefits for the implementation of various biomedical studies. The improvement of cell culture systems (especially 3D cell cultures, co-cultures) will increase significantly their predictive value and facilitate the translation of "*in vitro*" experimental data into results of importance to clinical practice.

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