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DIFFERENTIAL HEAT SHOCK RESPONSE OF PRIMARY HUMAN CELL CULTURES AND ESTABLISHED CELL LINES

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The influence of hyperthermia on the cellular growth and protein synthesis pattern from primary human brain tumour cells and skin fibroblasts was compared with established and experimentally transformed tumour cell lines. Primary cell cultures did not show any visible morphological changes after 42°C treatment, whereas in immortalized cell lines usually 90% of the cells were found in suspension. Enhanced expression of the major heat shock protein(hsp 70) was found in all heat-treated cells. In contrast to the primary cell cultures, established and transformed cell lines synthesized a protein with an apparent molecular mass of 70 kDa and an isoelectric pH of 7.0 as early as 3 h after the initial hyperthermal treatment.

It is the aim of the present study to compare primary cell cultures with a limited lifespan with established and experimentally transformed cell lines and their response towards experimentally induced stress. Primary cell cultures which lack or cannot express the genetically inherent factors responsible for immortalization may be helpful in gaining additional information about the cells response towards exogenously applied stress(1). One of the foremost differences between primary cell cultures and established cell lines is the capability of the latter for unlimited and vigorous growth.

That there is indeed a connection between immortalization of cells and heat shock proteins has been shown earlier(2). The adenovirus E1A product which has the ability to immortalize cells in culture is also responsible for the cell cycle control of the hsp 70 expression, the best characterized heat shock protein so far. Infection of mouse cells with SV40 and polyoma virus leads to the synthesis of two host proteins with mol.masses of 92 and 72 kDa, respectively, which were also inducible by 43°C treatment(3). Similar observations were made by Tsukeda et al.(4). Furthermore

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it was shown(5) that transformed cells are consistently more sensitive to 43°C exposure than control fibroblasts.

In this report we extend the observations on heat-induced morphology changes of primary cell cultures and established cell lines and report for the first time on the synthesis of a heat shock protein which is unique for established cell lines, since it was never found in primary cell cultures.

MATERIAL AND METHODS

Fibrosarcoma cell line HT-1080 is commercially available(Flow.Lab). The established and experimentally transformed glioblastoma cell lines HeRo and HeRo-SV were derived from tumour biopsies and studied after 60 days in subcultures(for details see Fischer et al.(6). SV80(a SV40-transformed Fanconi cell line) was a gift from Dr.E.Spindler,MPI fuer Biologie, Tuebingen,FRG). Biopsy material from the human brain tumours and from skin(scalp) was from the same patients(kindly provided by Prof.Loew, Dept.Neurosurgery,Homburg,FRG). In order to establish the different cell cultures the tissue pieces were prepared as described earlier(7). After forming a dense monolayer the cells were trypsinized and subcultured. Prior to radioactive labeling or hyperthermal treatment, cells were starved for 16 h in a methionine-deficient medium(MEM) with the addition of 10% dialyzed calf serum(8,9). Cells were usually grown at a cell density of 5×10^4 to 10^5 cells per 2 ml in 25 cm^2 Falcon flasks. For hyperthermal treatment the flasks were placed in a 42°C sand bath which was kept at 42°± 0.5°C in an incubator. Radioactive label, 100 µCi/mmol(NEN) ^{35}S-methionine, was added per Falcon flask and the incubation continued for 16 h. For all heat shock experiments controls were run in parallel at 37°C. After radioactive labeling, the medium was removed and the cells were washed twice with 1.5 ml washing buffer (140 mM NaCl, 7 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM Na_2HPO_4, 25 mM Tris-HCl,pH 7.4). Nuclei and mitochondria were removed by centrifugation at 30,000 x g for 15 min, 4°C(10,11). The postmitochondrial supernatant was adjusted to 130 mM KCl and was then centrifuged at 40,000 rpm for 3 h in a MSE 10x10 rotor. The ribosomal pellet was dissolved and stored in liquid nitrogen. The ribosome-free supernatant, material sedimenting slower than 30S, was diluted with five volumes of ice-cold acetone. Precipitation of the proteins was at -20°C overnight. The acetone precipitate was collected by centrifugation in 30 ml Corex tubes, 10,000 rpm, 10 min, 4°C in a Beckman Superspeed J2, dissolved in IEF sample buffer and analyzed by IEF 2D PAGE(7).

RESULTS AND DISCUSSION

Hyperthermia and cell morphology. Cells were exposed to 42°C for up to 16 h and compared to control cells maintained at 37°C(Fig.1). In the case of the fibroblasts and primary human tumour cell cultures no distinct differences in cellular morphology were observed before(Fig.1a) and after heat treatment(Fig.1b). Viable cell number was usually 90% as determined by Trypan Blue exclusion. Cells grown at low density were also

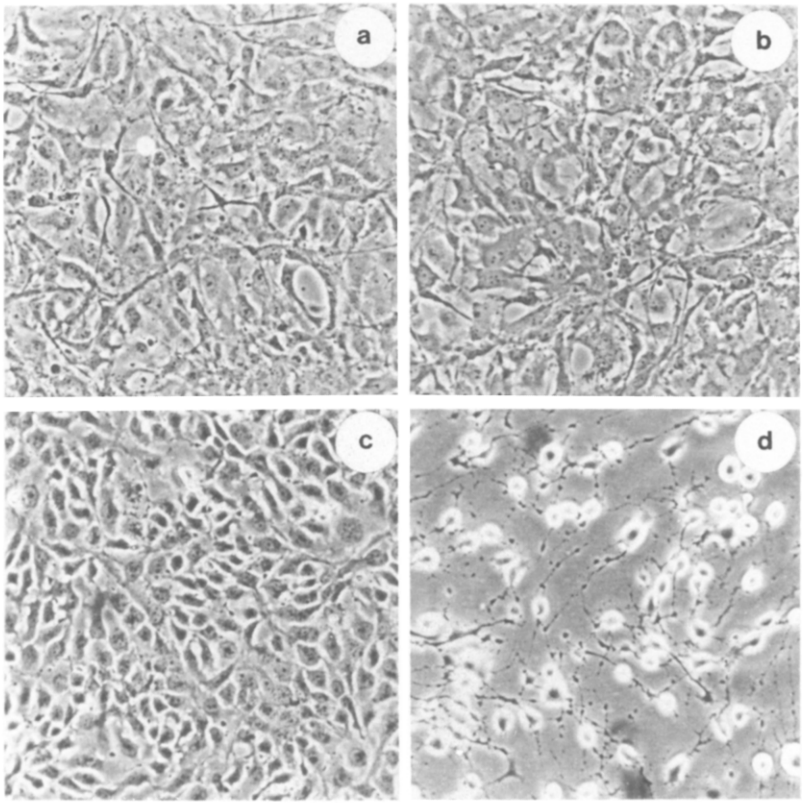

Fig.1. Morphology of control and 42°C-treated cells. Cells were photographed after 3 h of exposure to hyperthermal conditions. Primary tumour cells from meningioma biopsy, T2300 at 37°C (a) and at 42°C (b); SV80 cells at 37°C (c) and at 42°C (d).

indistinguishable when compared before and after heat treatment(results not shown). In contrast to these results all permanent and experimentally transformed cell lines investigated showed a clear reaction as early as 3 h after the initial heat shock, leading to a separation of the cells from the culture flask(Fig.1 c,d). In some cases up to 90% of the cells were found in suspension(Fig.1d).

Influence of hyperthermia on the protein synthesis pattern in primary cell cultures and permanent cell lines. The rational for our investigations concerning the protein synthesis pattern in primary cell cultures, established and experimentally transformed cell lines at 37°C and 42°C,respectively, stems from earlier observations where it was shown that primary

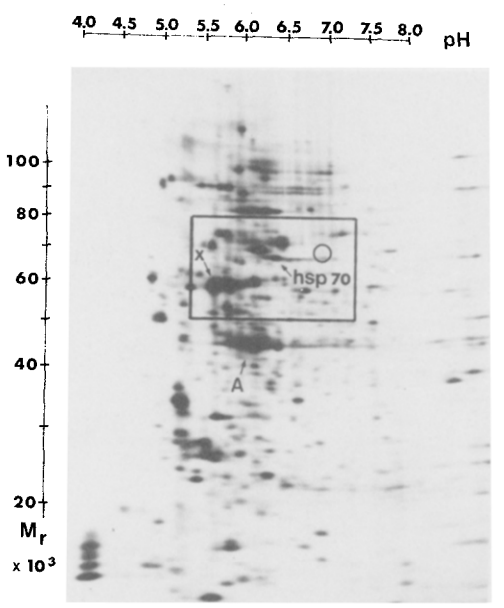

Fig.2. Protein pattern after IEF 2D PAGE analysis. Two-dimensional isoelectric focusing gel electrophoresis of extracts from cells at 37°C. Labeling of the proteins in the presence of ^{35}S-methionine was carried out as described in the Material and Methods section. About 2 x 10^6 c.p.m. of TCA-precipitable radioactivity was applied in a 20 µl volume in IEF sample buffer. Exposure for autoradiography was for 4 days. Analysis was by two-dimensional gel electrophoresis consisting of isoelectric focusing in the first dimension (right to left) and SDS gradient gel analysis (10-20%) in the second dimension (top to the bottom); A (actin), and X, a randomly chosen protein for reference purposes.
The position of the T70 protein which is not seen here, is outlined by a circle. The outlined area is further detailed in figure 3.

cell cultures and immortalized cell lines differed from each other in morphology, proliferation kinetics(6), casein kinase II activity(12) and with respect to the effects of tumour promoter TPA(13). Fig.2 shows an autoradiograph of total cytoplasmic proteins from the glioblastoma cell line HeRo at 37°C. The outlined area is further detailed in subsequent figures. The primary cell cultures and cell lines investigated show little or no qualitative differences in the overall protein synthesis pattern when grown at 37°C (Fig.3 a-f); yet there was a considerable change after hyperthermal treatment(Fig.3 g-m). The most prominent difference visible is the enhanced synthesis of the already well known major heat shock protein hsp 70. In addition to the observed hsp 70 increase a new protein which we call T 70 with similar mol.mass, albeit higher isoelectric pH appears in 42°C treated cell lines(Fig.3 i-m) and which

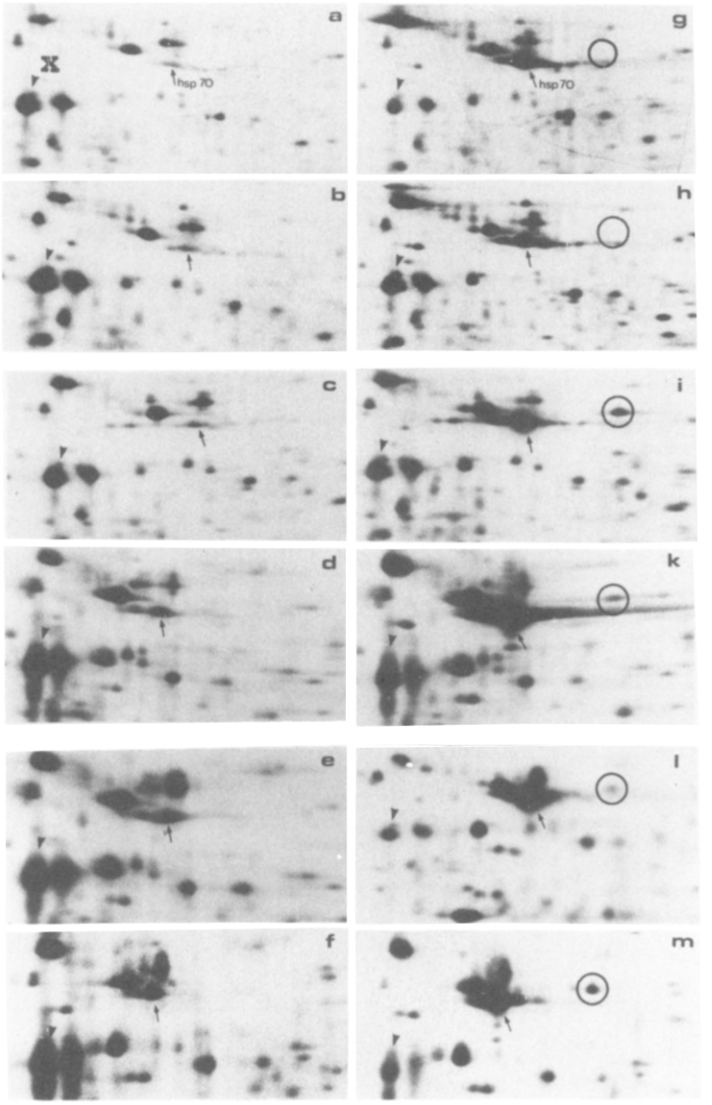

Fig.3. Autoradiographs of ^{35}S-methionine labeled cytoplasmic proteins from primary cell cultures (a,b) and tumour cell lines (c-f) at 37°C and at 42°C (g-m). (a) skin fibroblasts from meningioma patient T2300, (b) meningioma T2300, (c) fibrosarcoma HT-1080, (d) glioblastoma HeRo, (e) SV40-transformed glioblastoma HeRo, (f) SV80.
The corresponding protein patterns after 16 h at 42°C are shown in the photographs (g-m). The photographs are close-ups of the outlined area on figure 2.

is completely absent in primary cell cultures(Fig.3 g,h). We next asked whether there were significant quantitative differences between hsp 70 and T 70 synthesis in primary cell cultures and cell lines, respectively. Table 1 lists the total radioactivity associated with hsp 70 and T 70, actin and a randomly chosen protein termed X with

Table 1. Quantitative determination of the radioactivity associated with different protein spots after 2D PAGE analysis of cytoplasmic extracts from fibroblasts,primary tumour cells, established and experimentally transformed human cell lines

Cell cultures investigated	Temperature °C	hsp 70 c.p.m.	Increase -fold	T 70 c.p.m.	Increase -fold	Actin c.p.m.	Decrease %	X-Protein c.p.m.	Decrease %
Fibroblasts F2300	37	1,420	14	425	-	23,100	63	13,150	68
	42	23,800		270		8,300		4,050	
Meningioma T2300	37	1,300	7	427	-	17,000	20	8,700	52
	42	11,000		350		13,600		4,000	
HT-1080	37	3,000	25	450	9	12,000	32	20,630	26
	42	84,500		6,576		8,100		15,260	
SV80	37	1,180	20	390	3.6	13,100	24	20,400	54
	42	29,800		2,170		11,347		9,270	

The blank value(300 c.p.m.) was determined by excising a piece of gel from an area where no radioactivity was detectable by means of autoradiogrpahy after four days of exposure. Blank value was substracted from the numbers shown in the table.

hitherto unknown functions. Maximum increase of hsp 70 synthesis was approximately 20-25-fold in the established cell lines SV80 and HT-1080, respectively and varied from 7-14-fold in primary cell cultures. Protein T 70 was stimulated between 3-9-fold in the established cell lines and not at all in primary cell cultures. Indeed,a reduction in the T 70 synthesis appears to occur(Table 1). Together with the onset of the enhanced hsp 70 synthesis a general decrease in the overall protein synthesis pattern was observed after applying stress conditions. We have chosen actin and another protein with unknown functions, here marked X as a reference to determine the percent decrease during heat shock conditions. The average decrease of actin and the X-protein varies between 25 to 70%. This is in full agreement with reports from other laboratories so far as the decrease of common cytoplasmic proteins under stress conditions is concerned(14).

We can thus distinguish two categories of proteins when heat shock conditions prevail (i) proteins that are enhanced under stress conditions in all types of cells, whether primary tumour cells or permanent cell lines(the so-called heat shock proteins) and (ii) proteins that become shut off when stress prevails(the majority of cellular proteins). Heat shock protein T 70 seems to belong to a third category of proteins that are synthesized under heat shock exclusively in immortalized cell lines and not in any of the primary cell cultures examined.

As we pointed out earlier, thus far a direct comparison between primary cell cultures and established cell lines has not been carried out. This might account for the previous absence of characterization of this molecule as a heat shock protein. T 70 protein is not produced in large amounts less than 10% as much radioactivity as is associated with hsp 70 is found to be incorporated into T 70 (Table 1). Furthermore T 70 is neutral (pI 7.0) whereas all other heat shock proteins are acidic (pI 5-6) (15,16) which may also account for the fact that T 70 was overlooked by 2D PAGE analysis. Recently, a novel heat shock protein was discovered in chicken embryo fibroblasts(17) which differed from most known heat shock proteins by its unusual pI.

The possible function of T 70 is open to speculation but since immortalized cell lines are more vulnerable to effects of hyperthermia, the synthesis of a novel heat shock protein might be another protective mechanism not required by the less sensitive primary cell cultures.

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