Melanogenesis influence on amelanotic melanoma cells biology

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Introduction

Amelanotic melanoma is one of the less known form of melanoma where cells do not produce melanin. Thus amelanotic melanoma is characterized by a lower level of cells differentiation, a higher growth rate, a higher malignancy and worse prognosis than the melanotic melanoma. Our earlier comparative studies with Bomirski hamster's transplantable melanoma: melanotic (Ma) and amelanotic (Ab) shown many biological differences e.g. different susceptibility to spontaneous and camptothecin-induced apoptosis. Ab line cells do not produce melanin but have an active tyrosinase, the basic enzyme for melanogenesis. In this study we would like to estimate how these cells cultured in a medium with high level of tyrosine, a substrate for melanin synthesis, will change their biology e.g. cell cycle, ability to spontaneous death.

Material

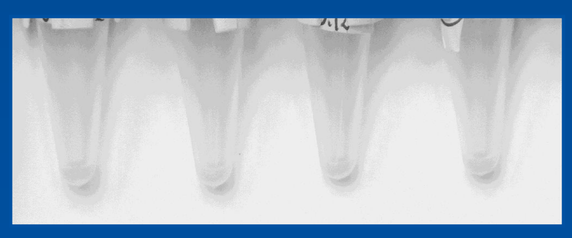
In our laboratory we use a model of Bomirski melanomas – two hamster transplantable melanoma lines of common origin: a melanotic (Ma) and an amelanotic (Ab). The Ab line originated from a melanotic form and the loss of pigment was accompanied by changes in many biological features. Melanoma cells were isolated from solid tumors by a non-enzymatic method after 10-12 days of Ab melanoma growth. Cells were cultured in two media, RPMI and DMEM (high tyrosine level) suplemented by 10% FBS and antibiotics for 12, 24, 36 and 48 hrs.

Method

Cell cycle analysis was estimated by staining DNA with propidium iodide (PI). The activity of caspases (basic proteases in apoptosis) were analyzed by FLICA test (Flurochrome-Labeled Inhibitors of Caspases; FITC-VAD-FMK; Promega) and esterases (hydrolytic enzymes) by CFDA (Carboxyfluorescein Diacetate, Molecular Probes) as a substrate for esterase.

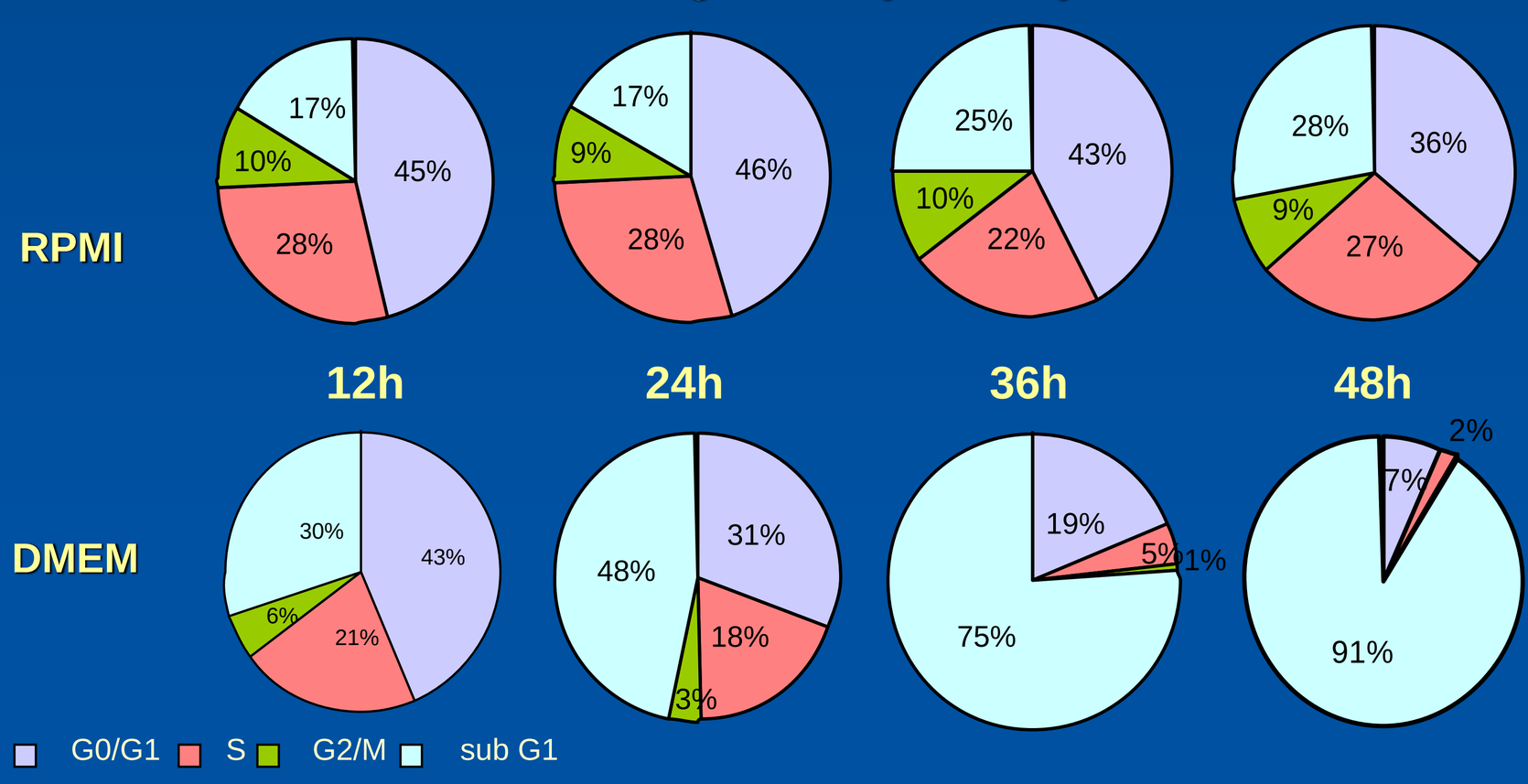
Results

Melanization



Cells cultured in DMEM doubled the melanin content after 24 hrs and during additional time it still increases.

Fig 1. Cell cycle analysis



The content of cells from all cell cycle phases decreases with the melanin production. After 24 hrs in DMEM about 50% of cells are located in sub G1, which includes damaged cells with low amount of DNA and the apoptotic bodies (Fig.1).

Fig. 2. Cells with activated caspases

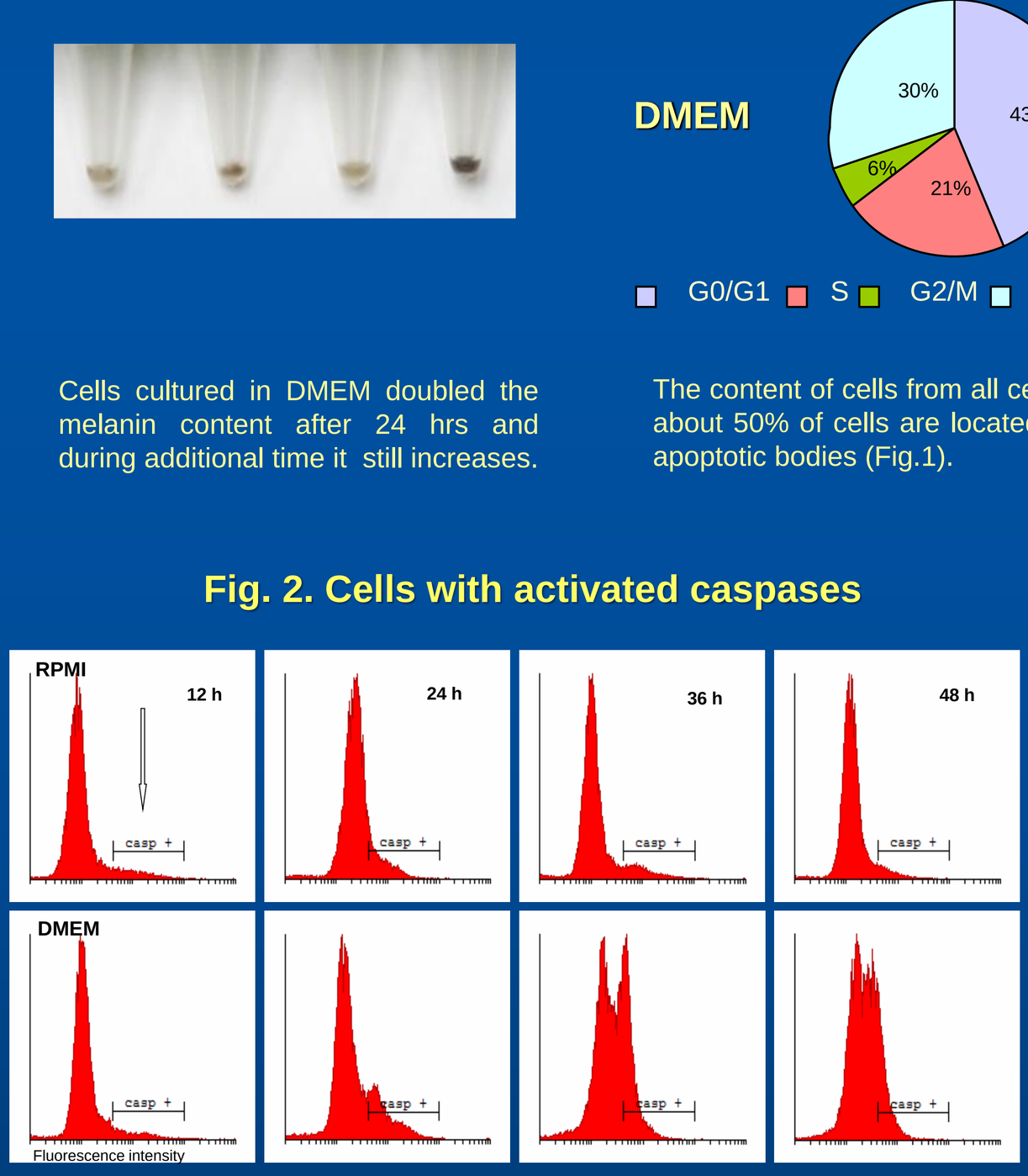
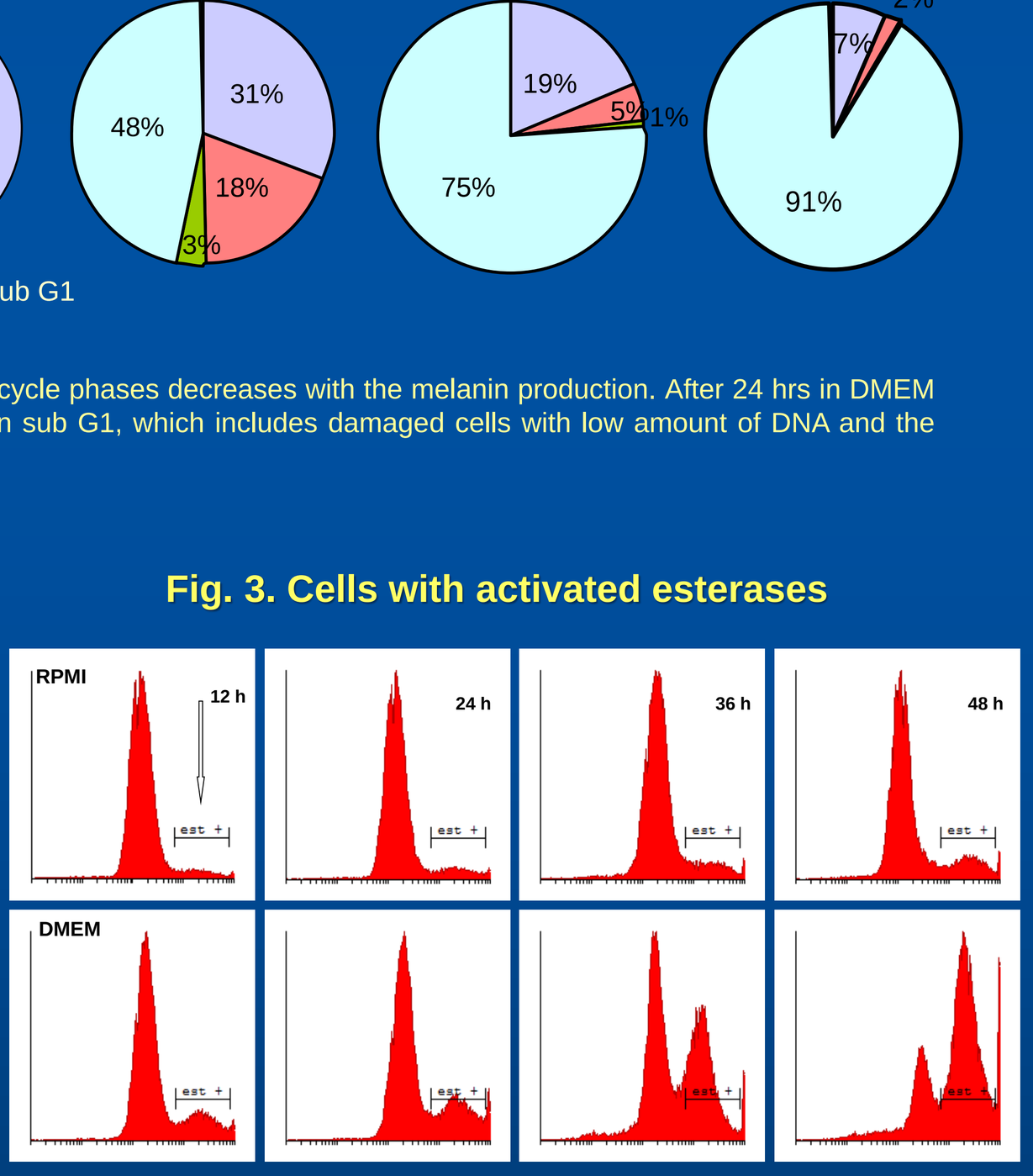


Fig. 3. Cells with activated esterases



After 24 hrs incubation in DMEM the content of cells with activated caspases and esterases doubles in comparison to incubation in RPMI. During prolonged time incubation we observed increasing number of cells with activated esterases; after 48 hrs over 60% of cells have activated esterases (Table 1, Fig.2 and 3) .

Percentage of Ab cells					
Medium	**Measurements**	**12h**	**24h**	**36h**	**48h**
RPMI	**G0/G1**	44,7 ± 7,7	**44,8 ± 6,1***	**41,4 ± 3,0***	**35,5 ± 3,1***
	S	27,3 ± 5,3	28,1 ± 3,6	**21,4 ± 5,3***	**27,0 ± 3,4***
	G2/M	**9,3 ± 2,2***	**9,0 ± 2,1***	**9,8 ± 3,0***	**8,5 ± 1,8***
	sub G1	**16,3 ± 4,8***	**16,8 ± 3,0***	**24,6 ± 5,9***	**27,2 ± 4,4***
	active esterases	9,77 ± 4,8	10,29 ± 3,8	**18,26 ± 5,7***	**15,94 ± 2,8***
	acitive caspases	11,6 ± 2,9	13,4 ± 7,1	21,3 ± 10,3	13,4 ± 6,5
DMEM	**G0/G1**	42,6 ± 5,6	**30,3 ± 6,6***	**18,9 ± 3,5***	**7,0 ± 2,3***
	S	20,7 ± 2,4	18,2 ± 6,8	**4,5 ± 2,1***	**2,2 ± 2,1***
	G2/M	**5,4 ± 0,7***	**3,5 ± 1,5***	**0,9 ± 0,5***	**0,2 ± 0,2***
	sub G1	**29,6 ± 2,3***	**46,8 ± 6,1***	**75,5 ± 1,6***	**90,5 ± 2,4***
	active esterases	16,8 ± 4,5	23,7 ± 12,5	**46,6 ± 18,2***	**60,5 ± 29,4***
	acitive caspases	14,7 ± 4,8	25,0 ± 7,2	36,5 ± 11,2	23,4 ± 7,8

Table 1. The content of Ab melanoma cells with activated caspases and esterases and cell cycle analysis after incubation in media RPMI and DMEM differing in the content of tyrosine. Percentages ±SD Statistical analysis by Kruskal-Wallis test; *p<0,05

Conclusions

In the light of our above-mentioned observations we can conclude that the incubation of amelanotic Ab melanoma cells in a medium with high level of tyrosine (DMEM) stimulates melanin production but melanogenesis induces cells death with caspases and esterases activity.