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Possible role of markers synthesized during cancer evolution: I. Markers in mammalian tissues

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Various trigger molecules (carcinogens, antimitotics, antibiotics, hormones, etc.) may induce mammalian cancer DNA chain relaxation correlated with an *in vitro* increase of DNA synthesis and the enhancement of cancer cell multiplication *in vivo* (1). In contrast, particular substances which contract cancer DNA chains inhibit cancer DNA synthesis and prevent cancer cell multiplication *in vivo* (2). During cancer evolution, large amounts of α-feto-protein (AFP), carcinoembryonic antigen (CEA) and calcitonin, may be synthesized. AFP normally produced by liver cells may accumulate in breast cancer tissues (3) or in induced inflammatory lesions (4); it favours the accumulation of estrone in the new-born rat brain (5) and affects the multiplication of estrogen-sensitive cells (6).

The aim of the present work was to determine the effect of some embryonic antigens (markers) on normal and cancer DNA chain relaxation and DNA *in vitro* synthesis, in order to investigate their participation in the maintenance of the neoplastic state *in vivo*.

Materials and methods: Human AFP and CEA were obtained from the Atomic Energy Authority, France, and pig calcitonin, from Armour Lab., France. Before use they were dialysed against sterile distilled water for 2 h at 4°C. Healthy human tissues, i.e., colon, liver, spleen (three cases), and cancerous human tissues, i.e., colon, breast, kidney, neurocarcinoma (three cases), after excision from patients were kindly provided by the Curie, Necker and Cochin hospitals and the St. Jean-de-Dieu clinic, Paris; the Meudon-la-Foret clinic and the Val-d'Or clinic, Saint Cloud, France. DNA from each individual tissue as well as DNA from healthy monkey (*Macaca mulatta*) brain tissue (one case) was purified as previously described (1). DNAs of the same type were pooled. DNA chain opening (hyperchromicity) was measured by UV absorbance increase at 260 nm (10 μg of DNA in 1 ml of 10^{-2} M Tris-HCl buffer, pH 7.3), before and after addition of tested compounds (1). The results were expressed as percent increase of UV absorbance induced by a given compound. Conditions for DNA *in vitro* synthesis were as previously described (1, 2).

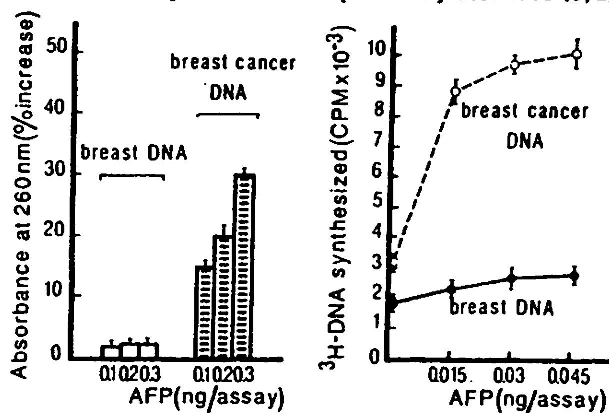

Figure 1: In vitro *strand separation of breast DNA and breast cancer DNA in the presence of AFP (left) and DNA synthesis* in vitro *(right) (means; bars indicate 1 SEM; n = 4 (left), n = 3 (right)).*

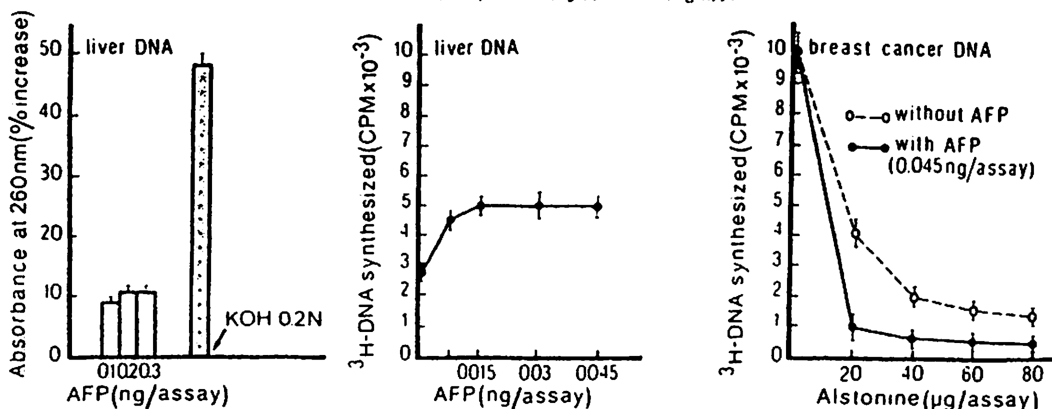

Figure 2 (left and centre): In vitro *strand separation and synthesis of liver DNA in the presence of AFP (means; bars indicate 1 SEM; n = 3).*
Figure 3 (right): Inhibition of breast cancer DNA in vitro *synthesis by alstonine in the absence or presence of AFP (means ± SEM; n = 3).*

Results and discussion: Figure 1 shows the extent of both healthy and cancer human breast tissue DNA chain relaxation and DNA *in vitro* synthesis in the presence of AFP. This antigen induced a strong UV absorbance increase essentially of breast cancer DNA. It highly stimulated the synthesis of this DNA since cancer DNA chain separation

provides more initiation sites for DNA-dependent DNA polymerase, thus allowing increased DNA synthesis. AFP had little effect on healthy breast DNA (Figure 1), and poorly interacted with healthy human colon, spleen and monkey brain DNA (Table 1). However, DNA from liver tissue, the major producer of AFP, underwent in the presence of this antigen a weak chain relaxation resulting in a slightly increased DNA synthesis (Figure 2). CEA, a cancer marker mostly synthesized by colon tissue (8), monitors the efficiency of therapy and prognosis of patients with cancer of the gastrointestinal tract, breast and lung (8, 9). Here, we have shown that it induces human colon cancer, breast cancer and neurocarcinoma DNA chain separation and enhances their synthesis (Table 1). In this respect, CEA exhibited a weak effect on DNA from healthy human colon and liver (Table 1). Monkey brain and human spleen DNAs were not sensitive to CEA. Calcitonin, a polypeptide used as marker for the prognosis of patients with cancer of the thyroid and kidney (10), induced human kidney and (surprisingly) breast cancer DNA chain relaxation (Table 2) and enhanced their DNA *in vitro* synthesis (Table 1). All three cancer markers used here exhibited a rather narrow specificity for cancer DNAs. However, AFP may internalize not only in some neoplastic cells (3) but also in induced inflammatory lesions (4). No explanation of its role in these tissues has been reported.

In contrast to DNA chain relaxation, contraction of cancer cell DNA (2, 11) may result in regression of cancer cell multiplication. For example, alstonine, a selective inhibitor of cancer DNA synthesis (2, 11) and of the multiplication of cancer cells (7), appeared to be more active in the presence of AFP, a destabilizer of cancer DNA chains (Figure 3). The effect of this alkaloid on cancer patients showing a high level of plasma AFP and CEA is under investigation. During chemotherapy (11) the level of serum markers decreases markedly, but temporarily, to a rather low value. However, it increases prior to tumour recurrence. This indicates a relationship between levels of markers and cancer cell development, yet it is not clear whether derepression of fetal genes is a compulsory event in the neoplastic transformation of cells.

Table 1: Effect of markers on in vitro *incorporation of* [^3H]*-TNP (cpm × 10^{-3}) into acid-precipitable material using different DNAs as template (means ± SEM;* n = 3*)*

Markers	Healthy tissues				Cancerous tissues			
	Colon	Brain[a]	Liver	Spleen	Colon	Neurocarcinoma	Breast	Kidney
No marker	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	2.7 ± 0.1	2.1 ± 0.1	2.9 ± 0.1	2.1 ± 0.2
+ AFP	1.6 ± 0.1	1.6 ± 0.2	2.3 ± 0.2	1.8 ± 0.2	3.6 ± 0.2	2.6 ± 0.1	9.6 ± 0.2**	2.2 ± 0.2
+ CEA	1.8 ± 0.2	1.7 ± 0.1	3.0 ± 0.1*	–	12.4 ± 0.2**	12.3 ± 0.3**	7.2 ± 0.3**	2.1 ± 0.2
+ Calcitonin	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	1.8 ± 0.1	3.1 ± 0.2	2.2 ± 0.1	11.8 ± 0.5**	4.5 ± 0.3*

[a]Monkey (Macaca mulatta) *brain DNA. Concentrations of markers/assay: AFP, 0.05 ng; CEA, 0.08 ng; calcitonin, 0.03 units. In comparison with the value with no marker,* p < *0.02; **0.01 (Student's t test).

Table 2: Effect of CEA and calcitonin on in vitro *DNA chain relaxation (% increase) in healthy and cancerous tissues (means ± SEM;* n = 3*)*

Markers	Healthy tissues				Cancerous tissues			
	Colon	Brain[a]	Liver	Spleen	Colon	Neurocarcinoma	Breast	Kidney
No marker	0	0	0	0	0	0	0	0
CEA	5.0 ± 0.5**	2.6 ± 0.1*	12.1 ± 0.7**	2.5 ± 0.2*	25.2 ± 4.5**	16.0 ± 2.8**	20.3 ± 0.9**	1.4 ± 0.2
Calcitonin	2.5 ± 0.2*	1.4 ± 0.2	0.8 ± 0.1	–	1.5 ± 0.1	2.6 ± 0.1*	21.5 ± 3.2**	16.5 ± 3.0*

[a]Monkey (Macaca mulatta) *brain DNA. Concentrations of markers/assay: CEA, 0.02 ng; calcitonin, 0.02 units. For comparison between DNA chain relaxation in the absence or presence of markers,* p < *0.02; **0.01 (Student's t test).

The data described here show that fetal antigens constitute a class of glycoproteins and/or polypeptides capable of binding preferentially to cancer cell DNA, of separating DNA chains, and thus of leading to the unscheduled DNA replication that is characteristic of the neoplastic state. The active participation of fetal antigens in the maintenance of the cancer state is further supported by data which show that plant Crown-gall tumour DNA relaxation induced by mammalian fetal antigens results in the acceleration of plant tumour cell development (12). We may then ask: does an obligatory relationship exist between fetal antigens and activation of cellular oncogenes in the process of neoplasia?

1. Beljanski, M., Bourgarel P. and Beljanski, M.S. (1981) *Exp. Cell Biol.*, 49, 220-231
2. Beljanski, M. and Beljanski, M.S. (1986) *Oncology*, 43, 198-203
3. Uriel, J., Villacampa, M.J., Moro, R. *et al.* (1984) *Cancer Res.*, 44, 5314-5319
4. Riepi, G., Fielitz, W., Vercelli, J. *et al.* (1985) *IRCS Med. Sci.*, 13, 515-517
5. Aussel, C., Laliberté, F. and Masseyeff, R. (1985) *Life Sci.*, 36, 479-484
6. Soto, A.M. and Sonnenschein, C. (1980) *Proc. Natl. Acad. Sci. USA*, 77, 2084-2087
7. Beljanski, M. and Le Goff, L. (1983) *IRCS Med. Sci.*, 11, 363-364
8. Gold, P. and Friedman, S.O. (1965) *J. Exp. Med.*, 121, 439-462
9. Nawrocki, T. and Richan, P. (1979) *Environ. Nouv. Med.*, 2, 40-46
10. Cunliffe, W.J., Black, M.M., Hall, R. *et al.* (1968) *Lancet*, 2, 63-69
11. Le Goff, L. and Beljanski, M. (1982) *IRCS Med. Sci.*, 10, 689-690
12. Maeyama, M., Tayama, C., Inone, S. *et al.* (1984) *Gynecol. Oncol.*, 17, 104-116
13. Le Goff, L. and Beljanski, M. (1986) *IRCS Med. Sci.*, 14, 811-812

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