DETERMINATION OF ER IN OVARIAN CANCER USING MONOCLONAL ANTIBODY TECHNOLOGY

G. B. NARDELLI - V. LAMAINA - M. DAL POZZO - G. L. ONNIS
Institute of Obstetrics and Gynaecology - University of Padua (Italy)

Summary: The Monoclonal Antibody technology has been used in 29 cases of ovarian cancer. The immuno-enzyme-assay detected positive levels of estrogen cytoplasmatic repector in 51.8% of the cases as well as in the nuclear (51.8%). Moreover, the ER/EIA technique screened positive levels of Total ER (ER/t) in 72.4% versus 65.5% obtained by DCC-method.

24.1% of the cases had negative ER/t levels observed by EIA. The monoclonal antibody anti-ER is a very interesting method for studying hormone-dependent tissue, because it uses an immunological binding to antigenic protein (receptor).

The introduction of monoclonal antibody in a research procedure allows us to extend the " oncological know-how ". These antibodies bind the antigen-receptor independently of endogenous hormonal saturation of acceptor sites.

This is a guarantee in analyzing the results in oncology research and in ovarian cancer particularly, and so in our laboratory, we are trying many methods to identify the hormonal correlation in these tumors.

MATERIAL AND METHODS

Monoclonal antibody technology supplied by Abbott has been used in 29 ovarian cancer specimens in detecting estrogen receptors in the cytoplasm and in the nucleus (tab. 1).

We used this procedure when we could utilize only 0.3 ml of cytosol.

In EIA we used the Abbott system in a solid phase enzyme-immuno-assay based on the "sandwich" principle. Beads coated with M-Ab-Anti-ER from rat were incubated with 100 λ of cytosol in triplicate and were bound in the solid phase during incubation of 18 hrs overnight in a cold-room. Unbound materials present in the wells were removed by aspiration-washing with Pentawash II. M-Ab-Anti-ER from rat conjugated with horseradish-peroxidase was incubated with the beads at 37 °C/1 hr in Heraeus-B-5060-EK incubator. Unbound conjugate was removed by aspiration-washing. Then the beads were incubated with 300 λ of hydrogen peroxid and ortho-phenylendiamine-2HCl to develop color.

This reaction was stopped with 1 ml of 1 N sulphuric acid and each tube read in Quantum II at 492 nm. A STD-curve in quadruplicate was prepared simultaneously with the assay procedure and the protein was assayed according to Lowry's method. Results were expressed in fmol/mg.

Nuclear receptor

The pellets were resuspended in KCl-Buffer and homogenized with a teflon pestle directly in the polycarbonate tubes. They were then shaken gently for 30 min in cold-room on Varvel FVF-20 and afterwards they were centrifuged at 40,000 rpm/1 hr ([1]).

RESULTS

Table 2 shows the cytoplasmic and nuclear distribution of estrogen receptors assayed with immuno-enzymatic technique in ovarian cancer.

The anti-ER monoclonal antibodies were able to identify receptor concentration up to 10 fmol/mg (positive range) in a similar percentage (51.8%) of the cases (15/29) both in the cytoplasm and in the nucleus.

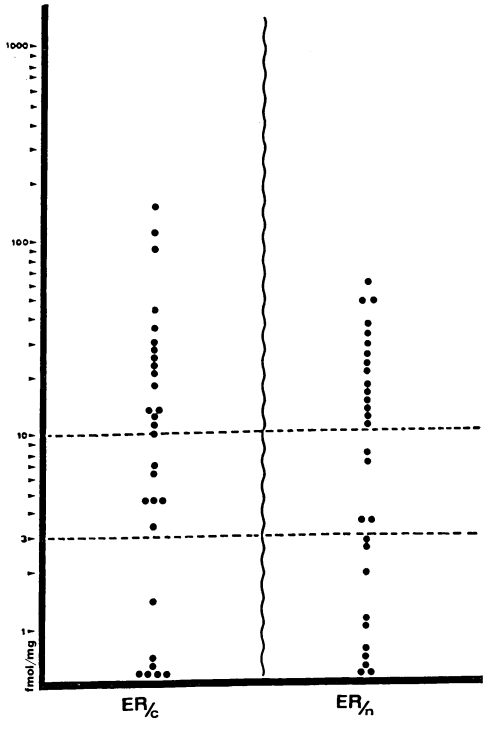
Regarding receptor concentrations lower than 10 fmol/mg, no significant variations in the borderline and negative ranges of the cytoplasm and nuclear compartments were observed.

In cytosol preparations 7 out of 29 cases had borderline detectable levels and 7 out of 29 cases had negative levels (less than 3 fmol/mg).

Table 1. – *Clinical characteristics and steroid receptor concentration of patients with different types of ovarian malignancy.*

Patient	Age	Menarche	Last period	Para	FIGO stage	E_2R/c EIA	E_2R/n EIA	Grading
			Serous papillary cystadenocarcinomas					
S. F.	51	13	45	3013	IV	0.38	0.33	G_2
M. B.	72	16	55	5005	III	14.8	8.35	G_2
T. C.	65	14	51	3013	IV	109	31.6	G_2
L. F.	56	14	54	0000	IV	3.6	2.9	G_1
M. P.	64	12	50	4004	III	14.8	23.9	G_2
P. C.	46	14	45	3003	III	35.9	12.3	G_2
E. T.	66	13	55	8018	III	19.2	38.2	G_2
M. B.	55	13	52	0000	III	28.7	16.5	G_1
C. C.	67	13	52	0000	III	12.5	2.8	G_2
M. F.	25	12	12 gg.	2002	I	91.6	68.1	G_2
G. M.	27	13	15 gg.	0000	I	1.05	1.30	G_2
L. A.	58	14	50	3003	III	23	11.5	G_2
R. T.	47	12	46	3023	III	0.15	0.74	G_2
A. U.	63	12	48	2012	III	0	0	G_2
			Mucinous cystadenocarcinomas					
G. P.	77	14	52	6026	IV	11	14	G_2
M. B.	79	11	50	0000	II	0	1.77	G_1
			Endometrioid adenocarcinomas					
F. N.	34	12	13 gg.	3003	I	6.6	20	G_1
D. M.	45	11	39	0000	IV	167	30	G_2
P. B.	37	14	5 gg.	0000	I	4.8	52.8	G_2
M. P.	46	14	12 gg.	2002	II	7.1	26.9	G_2
I. P.	69	13	50	3003	III	10	3.9	G_2
			Clear-cell adenocarcinomas					
M. C.	67	14	53	2002	III	22.4	2	G_2
P. B.	37	14	8 gg.	0000	I	4.8	52.8	G_2
			Granulosa tumors					
A. L.	59	12	45	0000	III	27.9	15.8	G_2
G. P.	43	14	42	1001	III	48.5	3.9	G_2
L. S.	52	12	49	2002	IV	4.8	7.8	G_2
R. P.	50	13	49	3104	III	0	0	G_2
			Arrhenoblastoma					
S. G.	49	14	9 gg.	2002	III	0	0.8	G_2
			Squamous carcinoma from a dermoid cyst					
E. P.	59	13	52	1001	IV	22.7	15.4	G_2

Table 2. — *Distribution of cytoplasmic and nuclear estrogen receptors (ER/c, ER/n) in malignant ovarian tissues.*



In the nuclear preparations 4 cases had borderline detectable levels and 10 cases had negative levels.

As regards the total estrogen receptor (ER/t) concentrations (table 3), the enzymatic-immuno dosage brought out a negativity of 24.1% (7/29) against a posisivity of 72.5% (21/29). The borderline cases were 3.4% (1/29).

These results show that the immunoenzymatic method is slightly sensitive as already pointed out in our research on mammary tissue [2].

Table 3. – *Positive, borderline and negative (Percentage of total estrogen receptors in malignant ovarian tissues).*

	ER/t	
+	72.5%	(21/29)
±	3.4%	(1/29)
–	24.1%	(7/29)

DISCUSSION AND CONCLUSIONS

The role of receptors in developing ovarian tumors in not yet known. The utility of immuno-enzymatic method in ovarian cancer research showed a total ER positive in 72.5% of the cases without significant change in respect to what we found with DCC method (58.8%).

The very high difference observed between ERc-EIA and ERc-DCC raised a question: Do the monoclonal antibodies anti ER detect the receptor or the number of the sites?

With DCC method the detection of the specific binding sites are certain, while with a pool of monoclonal antibodies only the upper part of the receptor, or the botton or both or even a great part of this protein can be assayed; probably in the answer lies the explanation of the overestimation.

BIBLIOGRAPHY

1) Nardelli G. B., Lamaina V., Siliotti F.: *Eur. J. Gynaec. Oncol., VII*, 3, 151, 1986.
2) Nardelli G. B., Lamaina V., Coronella M. L., Alessi A., Sandri A., Petrillo M. R., Siliotti F., Spandri P.: "Anticorpi monoclonali anti recettori estrogenici (ER-EIA) nei tessuti ginecologici". II Congresso Internazionale Italo-Ispano-Lusitano di Patologia Clinica. Ancona, maggio 1986.