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FACTOR II ACTIVATING ACTIVITY IN EXTRACTS OF TUMORAL NECROSIS FROM TWO MURINE BREAST ADENOCARCINOMAS. A. Blanco, R. Bonfil, O. Bustoabad, M. Lazzari. Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Buenos Aires, Argentina.

Increased deposition and lysis of fibrin, associated with malignant tissue, has led to look for activators of both the coagulation and fibrinolytic systems produced by tumor cells. We report the evidences of a procoagulant activity (PA) in the extracts of intratumoral necrosis from two experimental breast adenocarcinomas in murine model (BALB/c). The tumors have different metastatic capacity (MC). M3 without MC and MM3 with high MC. The addition of the extracts to: 1- Normal Plasma, 2- Deficient substrates in coagulation factors, 3- Purified fibrinogen (I), showed: 1- Shortening of the plasma recalcification time (PRT) and APTT, without modification on prothrombin time (PT), 2- Reduction of the PRT on deficient substrates in factors: VIII; VII; VII and X; V; V, VII and X; without modification on II deficient substrate, 3- No PA on I. Table:

| | Normal Plasma | | Deficient Substrates | | | | | I |
|---|---|---|---|---|---|---|---|---|
| | PRT | APTT | PT | VIII | VII | VII-X | V | V-VII-X | II | |
| M3 | 52s | 28s | 20s | 57s | 69s | 130s | 107s | 133s | 338s | >20m |
| MM3 | 62s | 34s | 20s | 65s | 74s | 139s | 113s | 136s | 350s | >20m |
| C | 96s | 51s | 20s | >10m | >10m | >10m | 397s | >10m | 360s | >20m |

C: Control, s: seconds, m: minutes. The PA was not affected by heparin. The results suggest that the PA is independent of the presence of either factor VIII or factor VII (intrinsic or extrinsic pathway respectively), as well as presence of either factor V or factor X. Any effect was observed either on factor II deficient substrate or on I, so, there was no evidence of thrombin activity The PA could be act directly on factor II, suggesting that fibrin formation could be induced by a "non-classical" activation pathway. No significant differences ($p>0.5$) in PA were observed between both tumoral necrosis extracts. The necrotic area in M3 (37%) is bigger than in MM3 (18%). So, much more PA could be present in MM3 and this could play a role in the MC of this tumor.

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ADHESION OF TUMOR CELLS TO EXTRACELLULAR MATRIX IS MEDIATED BY FIBRONECTIN. L.Almirall , J.Aznar-Salatti, I.Calopa, A.Ordinas, and E.Bastida. Hospital Clinic i Provincial. Facultad de Medicina. Universidad de Barcelona. Barcelona. Spain.

Tumor cell (TC) vessel wall adhesion is thougth to occur at specific sites of exposed extracellular matrix (ECM). To determine the role of fibronectin (FN) in TC/ECM adhesion, we measured; 1) TC adhesion to intact cultured endothelial cell monolayers (EC) or their ECMs, with or without incubation with a polyclonal antibody (Ab) to human FN, or a monoclonal antibody (Mab) to the cell binding site of FN (3E3); 2) TC adhesion to EC or their exposed ECMs incubated with specific peptides against bacteria adhesion sites (I 133-79) or the peptide GRGDSP contained in the cell binding sites of several adhesive proteins. TC adhesion was measured as number of $^{111}$In-labeled A-549 adenocarcinoma cells x10 /disc. ECMs were exposed by removing the ECs with $N_2$ flow or EGTA treatment. There was 5.5±1x10 A-549/ EC covered disc (Table). Treatment of ECs with the Abs or peptides had no effect on TC adhesion. TC adhesion to the two ECM preparation were 42±12 and 55±10 respectively. (Table). Blockage of the FN adhesive site by either Ab inhibited TC adhesion ($p < 0.01$). In contrast, blockage of the bacteria-adhesion site or incubation with GRGDSP had not significant effects in TC adhesion. The table shows the results (mean±SEM) (* $p < 0.01$).

| | A-459x10$^4$ cells/disc | | |
|---|---|---|---|
| Preincubation | EC | ECM($N_2$) | ECM(EGTA) |
| Buffer | 5.5±0.7 | 42±12 | 55±10 |
| Anti-FN | 5.3±1.0 | 29± 9 * | 34±11 * |
| 3E3 | 5.8±0.9 | 26± 3 * | 29±16 * |
| I 133-79 | 5.8±0.6 | 40± 6 | 48±10 |
| GRGDSP | 5.4±0.8 | 38± 7 | 50± 8 |

We conclude that TC adhesion to ECM but not to ECs, is dependent upon FN.

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FACTOR XIII AND ITS NATURAL PLASMATIC TARGETS: FIBRINOGEN, FIBRONECTIN AND ALPHA$_2$-ANTIPLASMIN IN MALIGNANT DISEASE. J.Kłoczko, M.Wojtukiewicz, M.Bielawiec, E.Pilecka Dept. Haematology, Medical School, Białystok, Poland.

The relevance of fibrin formation and its stabilization in tumour growth and metastasis formation seems to be important but it still remains unclear. The role of F.XIII in this process may emerge from a variety of mechanisms it is involved in, including crosslink of fibrin molecules, $\alpha_2$-AP incorporation into fibrin and reciprocal crosslink of fibronectin, fibrin and collagen. We studied F.XIII and its natural targets such as fibrinogen, fibronectin and $\alpha_2$-AP in 61 patients with various inoperable neoplasms (21 with Ca mammae, 18 with Ca ventriculi and 22 with Melanoma malignum). F.XIII activity was measured by dansylcadaverine incorporation method acc. to Lorand et al. F.XIII subunit "a" and "b", fibronectin, $\alpha_2$-AP, AT III, $\alpha_2$-M, $\alpha_1$-AT and $C_1$-I plasma concentrations were estimated by means of rocket immunoelectrophoresis acc. to Laurell using monospecific antisera (Behringwerke AG, Marburg). The following tests were performed in all cases: fibrinogen concentration, euglobulin lysis time and serum FDP. In comparison with healthy subjects the patients revealed statistically significant decrease in F.XIII activity and its subunit "a" and "b" concentrations concomitant with lowered fibronectin and $\alpha_2$-AP levels. No statistically significant correlations were found between F.XIII and its natural plasmatic substrates. Furthermore, the malignant patients showed decreased AT III concentrations, whereas $\alpha_2$-M, $C_1$-I and $\alpha_1$-AT levels were elevated. Prolongation of ELT concomitant with an increase of FDP concentration were also found. Differences in F.XIII level and in other haemostasis parameters were stated between different tumour types. Our data indicate that the role of F.XIII in malignancy is not limited to fibrin stabilization but its interactions with fibronectin and $\alpha_2$-AP should be taken into account.

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**PROTHROMBIN AND ANTITHROMBIN III IN PATIENTS WITH HEPATOCELLULAR CARCINOMA.** **G. Leone (1), V. De Stefano (1), R. Ferrelli (1), C. Barone (2), C. Garufi (2), B. Bizzi (1). Istituto di Semeiotica Medica (1) and Istituto di Clinica Medica (2), Università Cattolica, Roma, Italy.**

**Prothrombin (F.II) and antithrombin III (AT III) levels were measured in 11 patients (mean age 61 years) with hepatocellular carcinoma. F.II antigen (Ag) mean levels (Laurell) were 1.39±0.53 U/ml and F.II activity (Ac) (clotting method) 0.9±0.21 U/ml; AT III Ag mean levels (radial immunodiffusion) were 1.18±0.32 U/ml and AT III heparin cofactor (HC) (amidolytic method) 1.15±0.31 U /ml. In 5 patients F.II Ag was higher than 1.2 U/ml; no patient had F.II Ag lower than 0.8 U/ml (normal range 0.7-1.2 U/ml). F.II Ac was in the normal range in all patients. In 4 patients both AT III Ag and HC were higher than 1.2 U/ml; no patient had AT III Ag and HC lower than 0.8 U/ml (normal range 0.75-1.2 U/ml). Seven patients had a long history of liver cirrhosis and 2 of them showed AT III Ag and HC of 1.8 U/ml; one of these two patients had F.II Ag and Ac around 1.00 U/ml, whereas the other had F.II Ag 2.4 U/ml and F.II Ac 1.2 U/ml. In these two patients a preliminary more extensive study was performed. In both subjects AT III plasma crossed immunoelectrophoresis was normal in the presence and absence of heparin and AT III crossed immunoelectrofocusing (CIEF) showed a normal pattern of 6 peaks (pH 5.2-4.6) and two additional small peaks at pH 4.5 and 5.4. In the patient with increased F.II Ag the CIEF of plasma prothrombin showed a large peak with asymmetric branches at pH 5.2-4.9, as in the control, and a large additional peak at pH 5.9; after plasma absorption with $Al(OH)_3$, the F.II CIEF pattern showed only the abnormal peak. We conclude that in patients with hepatocellular carcinoma F.II and AT III are normal, independently of previous history of cirrhosis; moreover, in agreement with previous studies (N.Engl.J. Med. 310,1427,1984), an abnormal prothrombin, which we demonstrated characterizable by the CIEF, can be synthesized.**