Dedication

Milestones in the Field of Tumour Microenvironment – Contributions and Perspectives of Professor Isaac P. Witz

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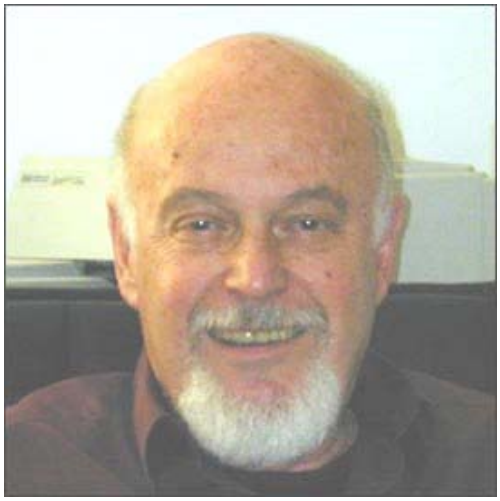
The development and maintenance of many cellular systems is tightly regulated by specialized microenvironments. Haematopoiesis and T-cell differentiation are well studied examples of this interaction. Likewise, tumour cells will respond to local stimuli generated by their microenvironment. These stimuli include cues for motility and migration, which normally appear in tissues undergoing formation, remodelling or healing. Microenvironment plays a critical role in the progression of tumours and may share responsibility in determining the "malignant" traits (invasiveness and metastasis) of these tumour cells.

As we are now celebrating the 70th birthday of Professor Isaac P. Witz, a leading and pivotal figure in the field of tumour microenvironment, this is an appropriate opportunity to review the milestones in this area, which parallel the scientific career of Professor Witz at the Department of Cell Research and Immunology at Tel Aviv University during the past forty years. Since 1967 Professor Witz has dedicated a major portion of his research career to investigating the "tumour microenvironment". It all began in studies on the role of the immune system in the defence against tumours. The rationale for initiating studies on "*in-situ* tumour immunity" was that "there is no reason to assume that expression of immunity is the same in all parts of the body" including the tumour site. Witz et al. showed that various types of *in vivo* propagating cancer cells in rats, mice and humans are coated with an "immunoglobulin (Ig) coat". This coat consisted of a mixture of tumour-reactive antibodies, undetectable in the circulation, and of unrelated immune complexes. In addition, immunoglobulins which totally lacked specificity to the tumour cells, and antibodies directed against membrane antigens expressed on normal and on tumour cells were detected. These findings led to a detailed study of the expression of Fc-receptors (FcR) on tumour cells. Such receptors were indeed detected on certain *in vivo* growing mouse tumours. Moreover, it could be shown later



Professor Isaac P. Witz

that the expression of FcR in FcR-positive cells was induced by microenvironmental factors such as INFγ. Surprisingly, transfection of FcγRII cDNA into FcR-negative tumour cells enhanced the tumorigenicity of the transfectants. Importantly, expression of FcR by tumour cells was lost and their tumorigenicity was attenuated following their adoption into culture. These experiments led to the conclusion that FcR exerted tumour-enhancing activity by delivering "malignancy-enhancing signals" to the tumour cells. Further studies conducted by Professor Witz and others established the role and implications of FcR tumorigenicity. Another set of studies was aimed at exploring the mutual interactions between tumour cells and microenvironment. For instance, it was shown that tumour-derived proteolytic enzymes were able to degrade Ig molecules in their microenvironment. This activity eliminated Fc-mediated functions of the antibodies such as complement-dependent cytotoxicity, and generated "blocking" Fab fragments that actually protected tumour cells from cytotoxic activity.

The concept that microenvironmental factors play an important role in cancer and in particular in metastasis has been around for a long time, but was overshadowed by the almost exclusive focus on cancer cell genomics. This has recently changed. The notion that the tumour

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microenvironment plays a crucial role in cancer development and progression is now widely accepted among cancer researchers. Indeed, recently Dr. Andrew C. von Eschenbach, Director of NCI, wrote in the Director's Corner of the NCI web site: "*The NCI identified the tumor microenvironment as a priority research area in an effort to expand our knowledge of the cells and factors that normally populate the microenvironment as well as to advance our understanding of how these microenvironment components interact with tumor cells.*".

Conclusive *in vivo* evidence that the microenvironment shapes the malignancy phenotype of mouse tumours was provided for the first time by the laboratory of Professor Witz. BALB/c 3T3 cells transformed *in vitro* (so that they never encountered an *in vivo* microenvironment in the transformed state) were either maintained in culture or passaged once *in vivo* and then returned to culture. *In vivo* passaged cells expressed a significantly higher malignant phenotype than the same cells that were kept in culture. This proved that cancer cells originating from a common clonal ancestor and having an identical genetic load manifested an entirely different degree of malignancy depending on their microenvironment. As Professor Witz concluded in his own words "by subjecting such cells to host-derived pressures during a single *in vivo* passage we are in a position to delineate between two types of cellular characteristics: those which are influenced by the transformation process and by culture-associated selective pressures on the one hand and those which are induced or selected by the host on the other hand".

As already mentioned, tumour cells expressed FcγRII only *in vivo* but not when the same cells were grown in culture. This suggested that *in vivo* operating factors (such as INFγ) took part in shaping the phenotype of tumour cells, i.e., the tumour-expressed FcR interacted with immune complexes and delivered "malignancy-enhancing signals" to the cells. Other tumour-bound molecules were also shown to be involved in "cross-talk" with microenvironmental factors. Among these were murine and human Ly-6 molecules, namely Ly-6 A/E and E48. For example, the interaction between tumour-expressed E48 and the corresponding microenvironmental co-receptors initiated a cascade of events in human head and neck squamous cell carcinomas (HNSCC). Signals transduced via E48 to HNSCC caused upregulation of the fucose-generating FX enzyme. This, in turn, caused an increased expression of fucosylated selectin ligands (such as sialyl Lewis-a) on the tumour cells, thus increasing their ability to adhere to E-selectin and to activate endothelial cells. Furthermore, transfection of FX cDNA to colon carcinoma cells increased their ability to adhere to endothelial cells and downregulated the expression of the FX enzyme. Moreover, transfection with small interfering RNA (siRNA) of FX caused downregulation of selectin ligands and diminished the ability of transfectants to adhere to endothelial cells and to E-selectin.

Tumour-derived chemokines and chemokine receptors expressed by tumour cells provide another means of communication between tumour cells and the microenvironment. Mammary tumour-derived MCP-1, for example, interacts with MCP-1 receptors expressed on monocytes. It was shown that such interaction attracts the latter cells to the tumour site and upregulates TNF-α secretion. This, in turn, upregulates the secretion of pro-malignancy factors such as IL-6, certain matrix metalloproteinases (MMPs) and MCP-1 by the mammary tumour cells, thus creating a "vicious cycle" of tumour microenvironment interactions. Conversely, and as another example for such interactions, is the CXCR4/SDF-1 interaction in which chemokine receptors expressed by tumour cells enable the interaction of such cells with microenvironmental chemokines. Witz et al. found that human neuroblastoma cells express CXCR4 and as such migrate to the corresponding chemokine ligand SDF-1. This interaction plays a role in the selective migration of tumour cells to specific metastatic sites. It was demonstrated that high and low CXCR4-expressing neuroblastoma variants show a different expression pattern of several metastasis-associated genes.

Still, another example for influence of microenvironment on tumorigenesis, provided by Witz et al., is the development of secondary leukaemia in mice whose plasmacytoma regressed as a result of melphalan chemotherapy. Secondary leukaemia developed only in melphalan-treated plasmacytoma regressor mice but not in normal mice treated with melphalan. These results supported the hypothesis that microenvironmental factors operating *in vivo* in the plasmacytoma regressor mice, such as a cytokine imbalance, created permissive circumstances for induction of secondary leukaemia. Similarly, recently Witz et al. utilized a gene expression array to identify genes that were differentially expressed in leukaemic and in remission phase leukocytes derived from human acute leukaemia patients. It was shown that the expression of the phosphatase Pyst2-L is significantly higher in the leukaemic rather than in the remission phase. Further studies showed that this phosphatase plays an important role in various signalling pathways and that the expression of this enzyme can be modulated by microenvironmental factors.

The following "postulations" can be surmised from Professor Witz's legacy:

The interaction between cells and their "neighbours" (e.g. endothelial, stromal or any other cells) may induce phenotypic alterations and exert reciprocal effects on both dialogue partners.

Each of the dialogue partners can produce membrane-bound/soluble molecules that may influence the cell itself and/or other nearby cells, in an autocrine/paracrine manner. Such interaction may induce/reduce signal(s) leading to alteration in the cellular phenotype of both cells.

Molecules in the microenvironment can induce/reduce signalling in any potential nearby cell, as long as the molecule can interact with a compatible receptor on self/target cell and/or enter the cell.

These "postulations" highlight the fact that cancer cells have the potential of "**doing anything**", depending on the combination of (1) "class I cancer genes", i.e., their genetic background, (2) "class II cancer genes", i.e., those genes whose expression was influenced by the microenvironment, and (3) the "congenial soil" they are located in, as already stated (Stephan Paget, 1889) in the "*seed and soil theory*": "*When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil.*". Putting together the above information and many recent publications by Professor Witz and others, it seems safe to conclude that the cancer cell itself is only one partner in the story of cancer development. The cancer cell interacts with its local and systemic microenvironments, and each profoundly influences the behaviour of the other. These tumour-host interactions permit, and even encourage, cancer progression. And as Dr. von Eschenbach wrote recently in the Director's Corner of the NCI web site: "*Greater knowledge of the tumor-host relationship will considerably improve our efforts to effectively prevent, detect, and treat cancer, and will bring us ever closer to the day when people can live with - and not die from - this disease.*".

In addition to his scientific achievements, Professor Witz encouraged research activities in the field of tumour microenvironment by organizing many international multidisciplinary seminars, workshops, forums, and conferences. In these events, the pivotal issue, i.e., the tumour microenvironment, was approached and discussed thoroughly by biomedical experts in the field. An international conference devoted to the entire spectrum of "tumour microenvironment" (Tiberias, Israel 1995) was the impetus to establish the "International Cancer Microenvironment Forum" (ICMF). This forum was founded by an international body of about twenty cancer researchers from ten countries. At their last meeting (Spain, 2003), the founding charter members of ICMF decided to upgrade the forum into the "International Cancer Microenvironment Society" (ICMS) of which Professor Witz was elected as president. The society aims to become a driving force in the development of cancer therapies that would target microenvironmental factors, thereby shifting the balance of tumour-microenvironment interactions towards tumour and metastasis suppression. Professor Witz and his colleagues in the ICMS trust that this aim will be achieved by promoting international cooperation and by stimulating basic and applied research in topics linked to cancer microenvironment.

As his former student, I would like to express my deep gratitude to Professor Witz for creating that "congenial soil" for me and for many other students along these years and for his skillful guidance during the entire period of my training. His profound knowledge in all realms of biology, stimulating mind and acceptance of new and unusual ideas inspired his students to be open-minded to scientific innovations and stimulating new ideas. His personal involvement and encouragement have created the optimal scientific "micro-environment" for his entire lab. I wish Isaac (Itzik) many more fruitful scientific years.

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