150
TUMOUR DORMANCY IN BREAST CANCER

Romano Demicheli

Istituto Nazionale Tumori, Milan, Italy

The review analyzes the recent evolution of two paradigms related to the development of breast cancer metastases. The continuous growth model is required to yield to an interrupted growth model, the tantamount of which are episodes of "tumour dormancy". Primary tumour removal, usually considered as intrinsically beneficial, proves to be able to perturb metastatic homeostasis and to result, for some patients, in the acceleration of metastatic cancer spread. Paradigm evolution is supported by a growing body of findings from experimental models and is required to explain breast cancer recurrence dynamics for patients undergoing surgery without or with adjuvant chemotherapy.

Classical models that were proposed to explain breast cancer metastasis dynamics after primary tumour removal assumed the implicit hypothesis that tumours must always grow. The concept of uninterrupted growth failed to explain findings from both local and local plus distant recurrences. In particular, the bimodal recurrence pattern, which presents an early peak at the second year after surgery, a second peak at about 5 years and a tapered plateau-like tail extending up to 15 years, should be explained. This pattern is independent of the seeded organ, and may be observed in all metastatic sites. On the contrary, a new dormancy-based model of metastasis development was found to better fit clinical data. According to this model, metastatic tumour may either continuously grow or even sojourn in two dormant states, *i.e.* single cells and avascular micrometastases, with orderly transitions between these two dormant states eventually resulting in progressive appearance of clinical metastases. Moreover, some precipitating event at the time of primary tumour surgical removal may have a triggering effect. In spite of a century of investigations, the effects of primary tumour surgical removal on metastases have practically been ignored by clinicians. Single cells may be induced to proliferate *via* the conversion of non-cycling G0 cells or by switching avascular micro-metastatic foci to active angiogenesis. These processes occur to different extent in pre- and post-menopausal patients. The model found confirmation by the analysis of the recurrence risk for patients receiving adjuvant CMF, where the recurrence reduction occurred at specific, temporally separate recurrence clusters at the first and third year, for both menopausal statuses.

The proposed dormancy-based metastasis development model implies some kind of control on tumour growth from the microenvironment, some kind of homeostatic effect upon distant metastases. These concepts are poorly understandable within the classical frame, where cancer is a genome-driven disease, *i.e.* a cell-autonomous irreversible process and where the tumour microenvironment is an idle bystander, sometimes forced to provide factors supporting tumour progression. However, all these views (epitheliocentric somatic mutation paradigm, irreversibility of the neoplastic phenotype, insignificant role of the microenvironment) have been challenged by experimental evidence both *in vitro* and *in vivo*. A new image of breast cancer is emerging. Cells that we label "cancer cells" go on with their peculiar ability to have cross-talk with the environment. This trait accounts for the neoplastic behaviour, ranging from quiescence to open growth, in different sites and/or at different times. Tumour dormancy and the counterintuitive consequence of primary tumour removal have a logical place in this context. The traditional image of breast cancer is changing.

151
PROTEOMIC ANALYSIS OF TELOMERASE INHIBITION BY TELOMERE SPECIFIC LIGANDS

Gabriel Mazzucchelli[1], Valérie Gabelica[1], Nicolas Smargiasso[1], Frédéric Rosu[1], Marie-Claire De Pauw-Gillet[2], Jean-François Riou[3] and Edwin De Pauw[1]

[1]Laboratory of Mass Spectrometry and [2]Histology and Cytology Laboratory; CART, GIGA, University of Liège, BAT. B6C, allée de la Chimie, 3, 4000 Liège 1, Belgium; [3]Regulation et Dynamique des Genomes, Museum National d'Histoire Naturelle, USM 503, INSERM U565, CNRS UMR 5153, Paris, France

Telomeres consist of protein complexes and repeated 'TTAGGG' double strand DNA sequences ended by a 3' single strand DNA of the same sequence. Progressive telomere shortening is observed *in vitro* upon cell divisions and with ageing *in vivo*. At a critical telomere length, shortened telomeres trigger a permanent growth arrest known as replicative senescence. Telomerase is an RNA-dependent DNA polymerase that extends telomeres by adding 'TTAGGG' repeats. It consists of a functional RNA component (hTR) which serves as template and a catalytic protein (hTERT) with reverse transcriptase activity. The expression of hTERT alone is sufficient for the immortalisation of cells. Telomerase is highly expressed in tumor cells but at very low level in most somatic cells. These observations make the telomerase an attractive target for anticancer strategies. One of these strategies relies on the use of drug candidates able to stabilize the particular telomere G-quadruplex DNA structures. The stabilization of these structures makes the telomere inaccessible for telomerase and thus inhibits telomerase activity.

The effect of the hTERT transfection was first studied on the proteome of human WI38 fibroblast cells (1). Then, the proteome alteration response of hTERT transfected WI38 cells

induced by the treatment of two G-quadruplexes ligands, telomestatin and TMPyP4, was analyzed. Both compounds can inhibit telomerase but have different selectivity for the different G-quadruplexes structures.

Proteome analysis of the treated cells reveals that TMPyP4 induces much more protein expression alterations than telomestatin probably due to its poor selectivity. TMPyP4 induces especially a drastic down expression of the hnRNPs, a modulation of the proteasome pathway, an apparent decrease of the translation and an over expression of several molecular chaperones. Telomestatin induces in particular an over expression of the protein BCL2A1 which is involved in drug-resistance of cancer cells and a probable increase of the translation. Both treatments have a common effect particularly on the molecular chaperone CCT (down expression), HSP90 alpha (over expression) and hnRNP D (down expression). The protein HSP90 alpha is also over expressed in hTERT transfected cells compared to parental cells. This protein is already a promising anticancer target protein due to its central role in oncogenesis and in telomerase activity regulation.

1 Mazzucchelli *et al*: Proteome Science *6*: 12, 2008.

152
LNCaP PROSTATE CANCER IMAGING WITH BIOLOGICALLY FUNCTIONALIZED GOLD NANOPARTICLES IN 2D AND 3D CELL CULTURES

D. Schol[1], M. Fléron[1], J.F. Greisch[1], M.C. De Pauw-Gillet[1], E. De Pauw[1], M. Jaeger[2], M. Frenz[2], S.A. Eccles[3], J. Bamber[3], S. Frosini[4], L. Masotti[4], M. Fournelle[5] and R. Lemor[5]

[1]University of Liège, Liège, Belgium;
[2]University of Bern, Bern, Switzerland;
[3]The Institute of Cancer Research, Sutton, Surrey, England;
[4]El.En. S.p.A., Calenzano, Italy;
[5]Fraunhofer-Institut fuer Biomedizinische Technik IBMT, St. Ingbert, Germany

A major challenge in oncology is to develop more accurate imaging assessments. The ADONIS Project intends to prove the concept of using optoacoustic imaging with biologically functionalized nanoparticles as an integrated biosensor based imaging system for the production of specific and sensitive data for accurate diagnosis of prostate cancer. This concept involves using contrast agents which upon photoactivation induce the local heating of their environment, generating pressure waves that are detectable by piezoelectric transducers.

One of the main objectives of this project is to produce and validate a versatile lab system composed of functionalized nanoparticles for diagnosis of different superficial and accessible cancers, *e.g.* prostate cancer. Gold nanorods have been synthesized and functionalized with antibodies targeting specific antigens on cancer cell lines. The foremost challenge consists in synthesizing rod-like nanoparticles absorbing about 1064 nm, the spectral range where biological tissues absorb the least. A wet chemical approach in solution, using surfactants as dynamic template and silver nitrate as growth inhibitor, is used for synthesis. Once the particles have been synthesized, the surfactant is replaced by a biocompatible polymer for use in *in vitro* tests. The polymer-coated nanoparticles are then coupled with an antibody directed against the cancer cells to guarantee the selective detection of the particles.

Prostate Specific Membrane Antigen (PSMA), a transmembrane protein considered as a suitable biomarker for prostate cancer, was selected as the primary target. Recognition and successful binding of the biosensor to PSMA is demonstrated by various techniques using cell monolayers and 3D cell cultures. PSMA localization on the LNCaP cell membranes was identified by immunocytochemistry (HRP, Q-Dots). Backscattered electron (BSE) microscopy and two-photon luminescence imaging proved that the biosensor is bound to the viable and fixed cells expressing PSMA.

Gold particles attached to cancer cells serve as contrast agents for optoacoustic detection. The concept of detecting PSMA-expressing tumours using this integrated optoacoustic biosensor system was confirmed on LNCaP spheroids (cell aggregates) in gelatine phantoms. This system is currently being tested on *in vivo* human tumour xenograft animal models and in the future will be tested on human tumour biopsies.

6th Framework Programme of the European Commission, STREP n° NMP4-CT-2005-016880.

153
CLINICAL AND BIOLOGICAL ASPECTS OF PERITONEAL MESOTHELIOMAS

Marcello Deraco[1], Nadia Zaffaroni[3], Federica Perrone[2], Dario Baratti[1], Raffaella Villa[3], Shigeki Kusamura[1], Genny Jocollé[2], Antonello D. Cabras[2] and Silvana Pilotti[2]

[1]Department of Surgery, Fondazione IRCCS Istituto Nazionale Tumori, Milano;
[2]Department of Pathology, Fondazione IRCCS Istituto Nazionale Tumori, Milano;
[3]Department of Experimental Oncology, Fondazione IRCCS Istituto Nazionale Tumori, Milano, Italy

Background: Diffuse malignant peritoneal mesothelioma (DMPM) is a rare and rapidly lethal neoplasm. In recent years, the combination of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (CRS+HIPEC) has resulted in a significant survival improvement, as compared to historical controls. Little is known about DMPM genetic and molecular features. In the present study, we assessed new prognostic indicators and therapeutic targets in a large series of DMPM undergoing CRS+HIPEC. *Methods:* From a prospective database of 86 cases, we selected 66 DMPM. Cases with well-