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NEW LEADS IN CANCER THERAPEUTICS: A KEYNOTE ADDRESS

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Abstract The main problems in cancer chemotherapy are related to the fact that the available drugs are not specific nor selective enough in their anticancer action. Therefore, even a low degree of resistance at the target tumor level is sufficient to impart clinical resistance because the dose of drug cannot be increased sufficiently to overcome it without incurring unacceptable toxicity. In the face of the above mentioned difficulties, several directions of research are being currently pursued towards developing more effective and selective treatments of cancer. These include: 1) continuing traditional approaches of drug discovery stemming from lead chemical structures and in many cases utilizing combinational chemistry followed by suitable screening efforts; 2) Increasing the antitumor effectiveness of available drugs through: a) making it possible to increase drug dose intensity by protecting normal tissues from limiting toxicity through genetic manipulation or combination with such agents as GM-CSF or IL15; b) attempting to increase the specificity of drug delivery through the administration of agents encapsulated in suitable liposome or conjugated with appropriate antibodies or cytokines; c) increasing the sensitivity of target tumor to a drug by specific metabolic modulations as it was done, for example, in the case of combinations of fluoropyrimidines with leukovorin; 3) counteracting resistance to drugs through genetic and/or epigenetic approaches aimed at modifying, for example, mechanisms of drug uptake or retention or at reducing anti-apoptotic mechanisms; 4) attempting to improve biotherapeutic treatments, for example, utilizing novel therapeutic vaccines or antibodies, or treatments based on intervention on angiogenesis or on intercellular or cell-matrix relationships; 5) continuing efforts to develop more effective and selective combination treatments with drugs, biologicals or different modalities; and, 6) developing new treatments based on intervention at novel molecular targets which have an essential role in the physio-pathology of the cancer cell. The latter approach is the main subject of this address.

Key words: molecular target based cancer chemotherapy, immunomodulation in cancer therapeutics, chemo-immunotherapy of cancer, polyamines and cell cycle inhibition

Resumen ***Nuevas orientaciones en la terapia del cáncer.*** Los principales problemas de la quimioterapia en cáncer están relacionados con el hecho de que las drogas disponibles no son específicas ni lo suficientemente selectivas en su acción anticancerosa. Por lo tanto aún un bajo grado de resistencia a nivel del tumor es suficiente para impartir resistencia clínica porque la dosis de droga no puede ser aumentada suficientemente sin incurrir en una inaceptable toxicidad. Dadas estas dificultades, numerosos proyectos de investigación están dirigidos hacia el desarrollo de tratamientos anticancerosos más efectivos y más selectivos. Estos incluyen 1) propuestas constantes para el descubrimiento de nuevas drogas sintetizadas químicamente seguidas de un apropiado monitoreo; 2) aumento de la efectividad antitumoral de las drogas disponibles a través de a) un aumento en la intensidad de las dosis pero protegiendo los tejidos normales de la toxicidad limitante a través de la manipulación genética o combinación con agentes tipo GM-CSF o IL15.; b) la administración de las drogas encapsulados en liposomas o conjugados con anticuerpos apropiados o con citoquinas; c) aumento de la sensibilidad del tumor a la droga mediante modulación metabólica específica; 3) contrarrestando la resistencia a drogas a través de propuestas genéticas y/ o epigenéticas; 4) intentando mejorar los tratamientos bioterapéuticos, utilizando nuevas vacunas terapéuticas o anticuerpos, o interviniendo en la angiogénesis o en las relaciones intercelulares o de la matriz celular; 5) desarrollando combinaciones de tratamientos con drogas más efectivas y selectivas o con diferentes modalidades y 6) desarrollando nuevos tratamientos basados en la intervención sobre nuevos blancos moleculares que tienen un rol esencial en la fisiopatología de la célula cancerosa. Este último enfoque es el principal tema de esta alocución.

During the past 40 years or so, cancer chemotherapy has achieved significant successes in the sense that at this time in many types of cancers complete tumor regression and long-term tumor-free survival can be induced by the use of chemical and/or biological treatments, particularly if these are given together and/or in combination with other modalities of treatment, such as surgery, radiotherapy, photodynamic therapy, or hyperthermia. Nevertheless, major obstacles still need to be overcome before chemo- and bio-therapies can provide effective

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generalized treatment of neoplastic diseases, particularly of most of the so-called solid tumors. The major difficulties are related to the fact that most of the cytotoxic agents available to date are not specific for target tumor cells and are not sufficiently selective in their antitumor effects; because of this inadequate selectivity, even a relatively small degree of natural or acquired resistance at the target cell level cannot be overcome by increasing the dose of drugs without incurring unacceptable toxicity. Although biological treatments can be relatively more specific in their antitumor action, to date they have proven to be rather ineffective, in part also because of the existence of diverse tumor escape mechanisms and in part because of practical difficulties in using proteins as therapeutics.

In the face of the above mentioned difficulties, several directions of research are being currently pursued towards developing more effective and selective treatments of cancer. These include: 1) continuing traditional approaches of drug discovery stemming from lead chemical structures and in many cases utilizing combinational chemistry followed by suitable screening efforts; 2) Increasing the antitumor effectiveness of available drugs through: a) making it possible to increase drug dose intensity by protecting normal tissues from limiting toxicity through genetic manipulation or combination with such agents as GM-CSF or IL15; b) attempting to increase the specificity of drug delivery through the administration of agents encapsulated in suitable liposome or conjugated with appropriate antibodies or cytokines; c) increasing the sensitivity of target tumor to a drug by specific metabolic modulations as it was done, for example, in the case of combinations of fluoropyrimidines with leukovorin; 3) counteracting resistance to drugs through genetic and/or epigenetic approaches aimed at modifying, for example, mechanisms of drug uptake or retention or at reducing anti-apoptotic mechanisms; 4) attempting to improve biotherapeutic treatments, for example, utilizing novel therapeutic vaccines or antibodies, or treatments based on intervention on angiogenesis or on intercellular or cell-matrix relationships; 5) continuing efforts to develop more effective and selective combination treatments with drugs, biologicals or different modalities; and, 6) developing new treatments based on intervention at novel molecular targets which have an essential role in the physio-pathology of the cancer cell; this latter direction of research aimed at the development of novel anticancer treatments is probably the most exciting one in this post-genomic era and holds promise to yield specific antitumor agents as well as a molecular basis for the establishment of individualized therapies. Ultimately, new treatments based on a molecular and genetic understanding of the carrier cell are expected to be applicable in chemoprevention strategies designed to benefit “at risk” patients currently being identified.

Anticancer treatments based on interference with novel molecular targets

A true explosion of knowledge has occurred in recent years on molecular mechanisms leading to the development of cancer or supporting the dysregulated phenotype of cancer cells. Thus, the molecular basis for multistep carcinogenesis, the function of tumor suppressor genes, the gene-dependent molecular mechanisms of apoptosis, the receptor and signal transduction functions required for the survival and progression of cancer and the alterations of the mechanisms of control of the cell cycle in cancer cells are only some of the areas where a great deal of new information has been obtained but indeed also where much further clarifications are still needed. In the light of what is known, however, it is now possible to formulate hypotheses projecting the exploitation of specific molecular targets for therapeutic intervention.

The role of a molecular target identified, for example, consequent to microarray visualization and chosen for therapeutic developments, needs to be validated in cellular and animal model systems; structure-based design of inhibitors or empirical high throughput screening of chemical libraries are pursued to identify lead compounds affecting that target; combinatorial chemistry is carried out to obtain compounds with increased effectiveness stemming from the lead structures; preclinical development of the new compound towards clinical trials follows accepted procedures but should include experimentation designed to further validate the target chosen for study and the hypothesis on which the drug development effort was based; early clinical trials should also include validation of the target in humans, in some cases through stratification of patients with tumors characterized by the presence of the functional target under study. The above mentioned approaches are general examples of investigations that are being pursued in many academic and industrial centers for the development of post-genomic, molecular target based, novel cancer therapeutics.

The selection of targets for drug development cannot be empirically random but must be based on information giving some credibility to a probable role the target might have in the life of a cancer cell. In general, the areas in which molecular mechanisms are evaluated as possible targets for drug development are: 1) gene transcription including the function and specificity of transcription factors and of the transcription machineries with emphasis on the protein-protein interactions involved in the formation of transcription complexes; 2) cell immortality factors and genomic instability including mechanism of DNA replication and repair as well as the role of telomerases; 3) post-transcriptional mechanisms of mRNA processing, stability and function as well as cellular antisense mechanisms of mRNA control; 4) signaling cascades including

receptor functions, cytoplasmic and nuclear signaling as well as cross-talks among signaling pathways; 5) cell cycle mechanisms of control including factors conditioning progression, the proper function of check points, and the mechanisms by which cells make decisions about their fate, for example to undergo differentiations, to die by apoptosis, or to proliferate; 6) factors affecting resistance to single or multiple drugs ranging from the expression of responsible genes, to mechanisms of drug uptake or retention, to changes in the target of drug action, to changes in DNA repair or apoptotic processes; 7) mechanisms of tumor angiogenesis including the production, metabolism and function of the factors and pathways involved as well as the role of the related receptors; 8) mechanisms unique to the metastatic process such as, for example, those concerned with invasion and/or attachment at sites of dissemination; 9) the complexities of tumor immunity including tumor-induced immunosuppression and tumor escape mechanisms, the role of cytokines as effectors and regulators, the mechanisms of antigen presentation, the cellular and humoral processes involved in antitumor action.

At this meeting some of the areas mentioned above will be discussed in detail and their potential critically evaluated. Briefly outlined below are a few examples that substantiate what was alluded to above.

Signal transduction inhibition

Many efforts are currently being placed in various Centers to develop inhibitors of specific signal transduction pathways[1, 2]. Thus, inhibitors of oncogene activation, for example of ras farnesyltransferase and specific inhibitors of receptor tyrosine kinases (RTK) and of protein kinase C (PKC) are already being tested clinically. Most of the small molecules that are being tested for inhibition of RTKs act at the ATP site and yet are relatively specific for one or another pathway. Natural or semisynthetic products as well as antisense oligonucleotides have been found to be specific inhibitors of PKC. Monoclonal antibodies to specific receptors have also been studied, for instance, those reacting with EGF-R or HER/neu. Some examples of inhibitors of EGF receptor signaling that are under clinical trial are Zeneca's orally active small molecule ZD1839 and Roche's monoclonal antibody Herceptin[3]; orally active small molecules inhibiting PDGF receptor signaling are Sugen 101 or Novartis GCP 57148 (STI 571); this latter compound is a potent inhibitor of bcr-Abl TK which in Phase I trials was found to induce objective responses in 100% of patients with bcr-Abl-positive CML[1,2,4]; these latter findings also provided proof of principle and validation for the hypothesis that specific signal transduction inhibitors may have effective antitumor activity in humans based on an inhibition of the target selected for drug development. Specific orally active inhibitors of VEGF-R have also been developed, for example, Sugen 5416 and Novartis CGP 79787 (PTK 787), which affect tumor angiogenesis.

Clinical trials of inhibitors of signal transduction may require Phase I trials which are different from those designed for the study of cytotoxic agents. In fact the endpoints may not be primarily the establishment of the maximum tolerated dose (MTD), which in many cases could not be reached, but would be drug-induced changes in validated targets of biological activity in tumors and/or in surrogate markers that could be measured in more accessible tissues, such as peripheral lymphocytes. It is also likely that this group of agents can be used most effectively in rationally designed combinations with other agents of this type or with conventional chemotherapy.

Another difficulty that may hamper the development of signal transduction inhibitors is the existence of "homeostatic" mechanisms, such as a measure of redundancy among certain pathways and, in some cases, "cross-talk" between pathways that assure tightly regulated control mechanisms involving protein-protein interactions. These may also offer new sites for novel therapeutic intervention.

Cell cycle regulation

The progression of cells through the mitotic cycle is carefully regulated basically by 4 sets of processes, namely cyclins-cdks specific interactions at different phases of the cycle, positive and negative phosphorylations by upstream kinases, the action of physiological cdks inhibitors, and the ubiquitination-mediated degradation of cyclin proteins and of additional protein factors.

Two main pathways have been found to be involved in the function of the G1/S checkpoint, which is one of the most studied cell cycle progression control sites. These are the so-called Rb pathways which yield the active E2F factors that activate genes encoding enzyme required for DNA synthesis in S phase and are therefore pivotal to the G1/S transfer, and the p53 pathway which yields several factors leading to apoptosis and also provides for the p16 and p21 inhibitors of the Rb pathway and thus of cell cycle progression. These two main pathways are intertwined through tightly balanced "cross-talk" which assure that the progression of cells through the cycle is well regulated and timely[5-7].

The inhibition of the Rb pathway by p16 and p21 and the consequent inhibition of G1/S transfer suggests that alterations of "cross-talk" processes can lead to inhibition or increase of cell cycle progression. Indeed attempts are being made to develop functionally active fragments of p16 or p21 as inhibitors of cell cycle progression. In another example, MDM2 is known to target p53 for cata-

bolic ubiquitination thus reducing both the p53-dependent inhibition of the Rb pathway and the p53-dependent mechanisms of apoptosis[8, 9]; based on this knowledge attempts are being made to develop peptides that would block the binding of MDM2 to p53 and thus would lead to stabilization of p53 and increased p53 function.

Interesting recent findings by Dr. Carl Porter and his group in our Department have shown that a specific polyamine analogue, DENSPM (N^1,N^{11}-diethylnorspermine), markedly inhibits the growth of melanoma and other tumors, that the degree of this inhibition is correlated with the induction of the polyamine catabolic enzyme Spermidine/Spermine-N-acetyltransferase (SSAT). The ensuing marked decrease of cellular polyamine pools is accompanied by a sharp G1 arrest due to p53 dependent increase of p21 and a related inhibition of Rb phosphorylation. Thus the discovery of the molecular mechanisms of action of this polyamine analog not only indicates the important role of SSAT in polyamine homeostasis, but also establishes a link between the polyamine requirement in cell growth and cell cycle control mechanisms[10].

Immunotherapy Approaches

Even within a cursory overview of current approaches in cancer therapeutics, it would be inappropriate to omit mentioning, albeit very briefly, some of the recent contributions of antitumor immunity to the development of novel and hopefully more specific anticancer therapies[11]. The transfer of immune lymphocytes, whether transfected or not with cytokines genes, or of antitumor antibodies, whether “humanized” or not, is a subject of continuous study and provides a basis for the development of new therapies, as is also discussed in this Symposium. The utilization of cytokines as effectors or regulators of the immune response towards instituting immunomodulation with antitumor potential is another important approach. The development of therapeutic vaccinations is being explored intensely also using modified tumor cells or tumor cell antigenic preparations or antigen activated dendritic cells. Therapeutically exploitable immunomodulation induced with combinations of relatively low doses of anticancer drugs with certain cytokines like IL2 or TNF has been a major interest of this laboratory; some of the studies carried out with Adriamycin (DOX) are briefly outlined below[12].

In syngeneic mouse model systems, it was found that DOX augments the activation of macrophages with consequent increases in killing capacity and increased production of TNF and IL1 which occurs by transcriptonal mechanisms; CTL responses to tumor cells are also increased as is the production of IL2 by T lymphocytes. A T down-regulatory cell is inhibited which is different in its phenotype from the precursors of T suppressor cells inhibited by cyclophosphamide. Stimulation of LAK cells activity and stimulation or inhibition of NK cells acting dependent on their location in spleen cells or PEC populations, respectively, were also noted in drug-treated mice. The agent also caused a selective killing of $CD3^-$ low $CD4^+$, $CD8^+$ thymic cells which may be involved in the drug-induced modulation of antitumor immune responses. Attempts were made to exploit the immunomodulating effects of DOX by giving the drug in combination with IL2 or TNF. After the administration of relatively low doses of DOX and prolonged administration of low doses of cytokines, curative effects were obtained in a syngeneic tumor model system in which as few as 10 tumor cells ultimately kill all the mice. These synergistic curative effects were seen both in a lymphoma and in a breast tumor model and are currently being verified in clinical trials. These studies showed that certain anticancer drugs are not necessarily immunosuppresive but can actually be utilized for their immunomodulating effects in cancer immunotherapy[11].

Conclusion

New sites of intervention for the development of novel anticancer therapies can be identified within specific mechanisms of cell control and through the characterization of novel gene products and their function. A target of a potential anticancer drug must be validated in pre-clinical and clinical studies. In some cases, appropriate surrogate markers must be developed to pursue optimal clinical trials that would respond to the novel requirements of molecular target oriented cancer therapeutics. With increased knowledge of mechanisms of regulation of the immune response, of antigen presentation processes, of tumor escape mechanisms as well as of tumor-induced immunosuppression, it seems now possible to develop immunotherapies of cancer that would be both effective against, and specific for, target tumor cells.

During this Conference several of the areas of potential therapeutic pursuit touched upon in this introductory address will be discussed in much greater detail by experts in the respective fields. It should be thus possible to assess more specifically the opportunities for exploitation of at least some of the approaches briefly alluded to herein and to visualize the potential impact of novel knowledge of the molecular biology of tumor cells and of new vistas in tumor immunology.

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LA PORTADA

Ricardo Roque Carpani. **Martín Fierro,** 1988
Acrílico sobre tela, 150 x 200 cm. Cortesía de la Comisión Nacional de Energía Atómica, Predio TANDAR, Centro Atómico Constituyentes. Presidente de la Comisión Organizadora de la Exposición Permanente: Dr. A.J.G. Maroto.

Ricardo Roque Carpani nació en el Tigre, Provincia de Buenos Aires, en 1930. En 1950 se radicó en Paris, inició estudios de pintura que continuó en Buenos Aires. Expuso por primera vez en 1957 junto con Juan M. Sánchez y M. Mollari. Fundó el movimiento Espartaco. Entre 1974/84 residió en España. Expuso en forma individual en numerosas ciudades de Europa y América. Su labor gráfica, ejecutada en su mayor parte para el movimiento obrero y organismos de derechos humanos constituye, junto a su actividad muralista, un aspecto destacado de su obra[1].

[1] *Extractado de:* Comisión Nacional de Energía Atómica. Artistas Plásticos con la CIENCIA, 101 Centro Atómico Constituyentes, Predio TANDAR, Buenos Aires, 1999; p. 110.