Conference paper
UDC: 616-006:618.11-006:616.348-006:616.351-006:615.277

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Molecular basis of future patients-tailored treatment

KEYWORDS: Pharmacogenetics; Antineoplastic Agents; Neoplasms; Ovarian Neoplasms

It has been long ago noted that the apparently identical tumors in different individuals do not respond identically to the same cytotoxic agents or cytotoxic agent combination.
The sensitivity or resistance of apparently identical tumors to cytotoxic therapy at that time has not been well explained. Different properties of cancer cells have been implicated in drug resistance or drug sensitivity, some of them inherent to cancer cells, some of them acquired during therapeutic manipulations.
In vitro tests have been developed in order to determine sensitivity of cancer cells to cytotoxic agents. These tests were based on culture of cancer cells in the presence of different cytotoxic agents and the rate of killing. Although in vogue some two decades ago, this technique did not achieve wide acceptance. One of the reasons for this technique failure is that a number of cytotoxic drugs are in fact pro-drugs requiring metabolic processing into active cytotoxic agents.
The new era of understanding the question of sensitivity or resistance has begun with the advent of biological agents and their introduction into human therapeutic arsenal for malignant disorders. The biological agents were found to act on different intracellular pathways related to inhibition of apoptosis. However, each different tumor type and even different tumors with apparently same histology were found to have their survival depending on non-identical pathways. It become clear that the use of biologicals might be a dead end if tumor cell survival pathways inherent to a specific tumor in a specific individual were not previously identified.
This was the first step to introduction of pharmacogenetics and pharmacogenomics into the practice of human oncology.
With our new developing knowledge of pharmacogenetics and pharmacogenomics the possibility has arisen that we could, on the basis of identification of subcellular plethora of enzymatics pathways, genetically determined enzyme and protein polymorphism and determination of critical antiapoptotic pathways in a given cancer cell predict sensitivity of a tumor to a given cytotoxic agent.
Some aspects are already been identified although a number of these data remain to be confirmed before being incorporated into clinical practice.
Two tumor types have been studied with this aim in mind, with different results: ovarian cancer and colorectal cancer.

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The manuscript was received: 30.09.2005.

Accepted for publication: 15.10.2005.

OVARIAN CANCER

Despite recent advances, ovarian cancer remains a major cause of cancer mortality in females. Organoplatinum compounds remain one of the mainstays in the treatment of this disease. In parallel with breast cancer an attempt of identification of novel targets for biologicals has been performed in order to advance this therapeutic area in the future.
The investigations have been carried out on AKT/mTOR system, Bcl-2, proteasome inhibition, CA-125, thyrosine kinaze inhibition and antagonizing EGFR.
We have some both clinical and experimental data.
AKT and mTOR phosphorylation has frequently been detected in ovarian cancer and these targets have been investigated in experimental models. It has been shown that 55% of ovarian cancers show elevated phospho-mTOR activity. Furthermore in 87% of ovarian cancers phospho-AKT staining was associated with active mTOR. It has been shown that ovarian cancers with high AKT activity can be modulated for augmented Cisplatin induced apoptosis, while those with low AKT activity proved not to be prone to modulation for augmented Cisplatin induces apoptosis. On the other hand, inhibition of mTOR activity with Rapamycin in AKT activity-rich cells resulted in G1 arrest; inhibition of mTOR activity with Rapamycin in AKT activity-poor cells produced no effect. So, mTOR appears as a potential target in a subset of ovarian cancers (1,2).
Sensitivity to Rapamycin might be predicted by determination of more assessable targets. Thus Bcl-2 expression might predict presence of other molecular markers of sensitivity to Rapamycin. Furthermore, downregulation of Bcl-2 appears to be useful in combination with Rapamycin. Thus Bcl-2 is another possible target for biologicals in ovarian cancer, as first step in enhancing cytotoxicity of organoplatinum compounds (3).
Some ovarian tumors have low growth fractions and thus are intrinsically resistant to conventional cytotoxic drugs. Solid tumor in humans are experimentally best studied on multicellular spheroids. The proteasome inhibitor P-341 proved to have potential to circumvent multicellular drug resistance and could be active against ovarian cancer with low growth fractions in vivo (4).
But, what do we have in clinical practice?
Oregovomab is an antibody against CA-125. Clinically it was well tolerated and induced an immune response with formation of circulating complexes. Trials for consolidation of clinical remission in patients with advanced ovarian cancer, however, demonstrated that Oregovomab did not improve the time to progression. Trials in salvage setting are ongoing (5).
The small molecule EGFR antagonist, Gefitinib, was tested in ovarian cancer. It was clinically well tolerated. The response rate in EGFR positive tumors was 9%. The general impression is that it has minimal activity in unscreened patients with recurrent ovarian cancer. Prescreening for EGFR mutations might improve response rate to Gefitinib (6).
Angiogenesis is pivotal in development and progression of ovarian cancer and Bevacizumab fared better. Activity was demonstrated mainly in small series of cases. Larger scale trials are planned (7).
HER-2/neu is overexpressed in 15-30% of ovarian carcinomas. HER-2/neu overexpression has been shown to be associated with increased risk of progression and death. No relationship was found between HER-2/neu score and histopathological features in ovarian cancer. Transtuzumab, an anti HER-2/neu monoclonal antibody was tested in refractory and recurrent ovarian cancer. The overall response rate was 7,3% and the median time to progression 2 months. It was concluded that the clinical value of Trastuzumab in ovarian cancer was limited by the low frequency of HER-2/neu overexpression and by low response rate among patients with HER-2/neu overexpression (8).
These data have implicated that a subcategory of ovarian cancer patients can be modulated for increased Cisplatin induced apoptosis by previous identification of relevant apoptosis related pathways. On the other hand these data have shown that antagonising CA-125 is not relevant to ovarian cancer cell survival. In contrast with breast cancer cells, identification of overexpression

of HER-2 neu in ovarian cancer is not of particular interest because overexpression of this parameter in a subpopulation of patients with ovarian cancer and its downregulation are not relevant for survival of cancer cells.

COLORECTAL CANCER

Until recently the only effective drug for colorectal cancer was 5-Fluorouracil, modulated by Leucovorin. Different 5-FU regimen were developed. These regimens were usually well tolerated although the main inconvenience was the infrequent but spectacular occurrence of non-hematological grade IV toxicities associated with bone marrow aplasia. This complication proved to be the consequence of systemic diphosphopyridindehydrogenase (DPD) deficiency. So, colorectal cancer was sometimes referred to as "monotonous cancer" since few modalities were available both for adjuvant treatment and treatment for systemic disease (9).
In the last decade the situation of metastatic colorectal cancer has dramatically changed with the advent of three new cytotoxic drugs: Capecitabine, Oxaliplatin and Irinotecan. The addition of three new active drugs in addition to 5-FU gave additional options for combination chemotherapy and sequential treatments that resulted with a significant prolongation of disease control and survival in patients with metastatic colorectal cancer (10-12). The impact of those drugs on survival and therefore their extensive use in clinical practice, associated with a non-negligible increase in the overall cost of treatment have raised the question of their rational use and of subsets of patients who would benefit most from their administration. The game was therefore moved to the field of pharmacogenomics and pharmacogenetics.
We now know that the new drugs such as Oxaliplatin and CPT-11 are more active in colorectal cancer than 5-FU in single drug setting either in relation to response rate and possibly survival benefit. But we also know that there are patients treated with 5-FU that achieve a long survival, in excess of 2 years. We also know that there are patients treated with either Oxaliplatin or CPT-11 who do not achieve disease control with these two drugs and whose best response is progressive disease.
So, the question has arisen whether we can or cannot predict efficacy of either of the three drugs by any means in order to individualize chemotherapy in a given patient in order to achieve the optimal result.
Pharamacogenomics and pharmagonetics are starting to give us a clue concerning this particular topic.

Molecular basis of the rational use of fluoropyrimidines

It appears that both tumor dihydropyrimidin dehidrogenase (DPD) and Thymidine synthetase (TS) are good predictors for 5-FU activity. A significant increase in TS expression score was observed in 5-FU sensitive colorectal cancers compared to 5-FU resistant ones (13). Although the role of DPD expression in cancer 5-FU sensitivity remained somewhat controversial it now appears that patients with low DPD expression have longer disease free interval or longer disease control with 5-FU than patients with high DPD expression (14). DPD expression in normal cells is a significant factor determining 5-FU toxicities, patients with DPD deficiency in normal cells tending to exhibit life-threatening toxicities when treated with 5-FU. However the DPD contend in normal and cancer cells in the same individual is not identical and there appears to be individuals with adequate DPD content in normal cells and low expression of DPD in cancer cells. By retrograde analysis it has been shown that patients with low tumor DPD and high tumor TS treated with 5-FU only can achieve survival of over 24 months (15).
The situation might not be identical with peroral fluoropyrimidines. UFT shares apparently the same pattern with 5-FU concerning cancer cell levels of DPD and TS (16). On the other hand, the S-1 compound (combination of Tegafur-CDHP and Potassium oxonate) is more active than 5-FU in cancers with a high DPD activity due the fact that CDHP is a potent inhibitor of DPD. Capecitabine could be presumed to be inferior to 5-FU in patients with low TP levels because TP is necessary for conversion of this pro drug into 5-FU that is its active principle (17); although we might expect a lower activity of 5-FU in colorectal cancer with low TP levels as compared to the same with high TP levels, the efficacy of Capecitabine in these patients would be even lower because there would be no conversion to active cytotoxic drug within cancer cells.
Thus it appears that, based on the tumor level of DPD, TP, and TS, we can make the choice between different fluoropyrimidines best suitable for a particular patient.

Molecular basis of the rational use of Oxaliplatin

Members of the glutathione S-transferase (GST) superfamily are important in cellular defense mechanisms. These enzymes attach reduced glutathione to electrophilic groups in a wide variety of toxic compounds, including chemotherapeutic agents. Certain polymorphisms in GSTs are associated with changes in enzyme activity, sensitivity to chemotherapy, and overall patient's survival. There are three subclasses of GSTs, designated as P-1, T-1, and M-1. The GST P-1 has been shown to be associated with slower or faster inactivation of Oxaliplatin in cancer cells and thus directly related to its activity concerning disease control (18).
There are three variants of GST P-1 differing in only one amino acid residue in the position 105. These three variants determine three phenotypes. The homozygous Isoleucin/Isoleucin phenotype, the homozygous valine/valine phenotype, and the heterozygous Isoleucin/Valine phenotype. This genetic polymorphism has been found to have a profound influence on disease control and survival in patients treated with Oxaliplatin.
In a retrospective study conducted on patients progressing on 5-FU and subsequently treated with Oxaliplatin the impact of genetic polymorphism of GST P-1 on the survival was analyzed. Patients homozygous for the Isoleucin/Isoleucin phenotype had a median survival of 7,9 months, whiled those homozygous of the Valine/Valine phenotype had a median survival of 24,9 months. The heterozygous patients, i.e. those of the Isoleucin/Valine phenotype had a median survival, which was intermediary i.e. 13,3 months.
Thus determination of the GST P-1 might have a crucial impact on choice of patients likely to respond to Oxaliplatin and to exclude from this treatment the ones that should have no benefit from it.

Molecular basis of rationale use of CPT-11

Although the results are still preliminary there appears to be a relationship between UDP-Glucuronosyltransferase (UGT) and activity and toxicity of CPT-11 (19,20,21).
The impact of the polymorphism of two members of this family, UGT1-A7 and UGT1-A9 was analyzed in relation to activity and toxicity. Low enzyme activity of the UGT1-A7 genotypes (UGT1-A7 2/2 and UGT1-A7 3/3) was associated with antitumor response and lack of severe gastrointestinal toxicity. In the UGT1-A9 family the UGT1-A9-118 genotype was significantly associated with reduced toxicity and increased response. UGT1-A1 and UGT1-A6 do not appear any impact on activity. However patients who are either homozygous or heterozygous for UGT1-A1- 28 appear to have a significant risk of toxicity by CPT-11.
So, it appears that determination of UGT1-A7 and UGT1-A9 polymorphism might predict at least toxicity to CPT-11 and perhaps enable us to select patients likely to have a good probability of response to CPT-11 without significant toxicities related to SN-38.

The good and bad prognosis patient with metastatic colorectal cancer

It is perhaps too early to speculate about the prognostic significance of molecular markers in predicting outcome of patients with metastatic colorectal cancer.
Perhaps, it is not.
We could conceive that a patient whose tumor has a high TS content, low DPD content in a patient who is homozygous for the Valine/Valine phenotype of the GST P-1 and with low enzyme activity of the UGT1-A7 should be a good

prognosis patient: its median survival on 5-FU could be over 24 months, on Oxaliplatin again over 24 months and this patient would have a good chance of having a therapeutic response to CPT-11 without excessive toxicity.
On the other hand a patient whose tumor has a low TS content and a high DPD content, homozygous for the Isoleucin/Isoleucin phenotype would have a poor chance of response to 5-FU and shall be probably resistant to Oxaliplatin. If this patient is in addition either homozygous to UGT1-A1- 28 he would have a significant risk for severe toxicity on CPT-11. This patient would be a classical poor prognosis patient even with the most recent drugs.

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