Challenging Drug Resistance in Cancer Therapy

Review of the First Nordic Conference on Chemoresistance in Cancer Treatment, October 9th and 10th, 1997

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The First Nordic Conference on Chemoresistance in Cancer Treatment was held in the Danish town of Helsingør on October 9th and 10th, 1997, under the auspices of the Nordic Cancer Chemoresistance Group (NCCG). The meeting focused on biochemical chemoresistance in a multidisciplinary approach. There were 19 oral and 15 poster presentations documenting recent advances in experimental and clinical research of drug transport mechanisms, DNA repair systems, detoxifying enzymes, drug target regulation, in vitro sensitivity tests, apoptosis inhibition, and strategies to circumvent chemoresistance. In the present paper we review the main issues that were addressed and discuss the findings with reference to the current literature in the field. The meeting demonstrated the plurality and the complexity of chemoresistance, which is a major obstacle to successful chemotherapy in cancer patients. The new insights to mechanisms of drug resistance and sensitization represent a useful basis for further development of strategies to circumvent chemoresistance in clinical practice.

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Resistance of cancer cells to chemotherapeutic agents (chemoresistance) is a major clinical problem and a frequent cause of treatment failure in cancer patients. Chemoresistance tends to involve multiple cytotoxic drugs simultaneously (multidrug resistance, MDR), and resistance to one agent often implies resistance to a series of structurally diverse agents. Gastrointestinal, hepatobiliary and renal cancer are largely unresponsive to chemotherapy and thus have a high degree of intrinsic chemoresistance, whereas leukemias, lymphomas, ovarian and breast cancers often respond to treatment initially, but acquire resistance during the course of the disease. Several factors may contribute to chemoresistance, including physical mechanisms such as deficient tumour blood supply, and biochemical mechanisms. The latter have been the subject of intense investigation for more than 25 years. Interestingly, one of the first reports on biochemical chemoresistance in cancer cells was made in Denmark (1), and initiated a flourishing scientific production by the Nordic academies in this research field. As a consequence thereof, the First Nordic Conference on Chemoresistance in Cancer Treatment was held in the Danish town of Helsingør on October 9th and 10th, 1997, under the auspices of the Nordic Cancer Chemoresistance Group (NCCG).

The first NCCG meeting focused on biochemical chemoresistance in a multidisciplinary approach. There were 19 oral and 15 poster presentations which presented recent advances in experimental and clinical research of drug transport mechanisms, DNA repair systems, detoxifying enzymes, drug target regulation, in vitro sensitivity tests and apoptosis inhibition (Fig. 1). To meet these challenges to cancer therapy, several presentations also focused on strategies to circumvent chemoresistance. In the present paper we review the main issues addressed at the first NCCG meeting with references to the current literature in the field.

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DRUG EFFLUX MECHANISMS

MDR is frequently associated with overexpression of the multidrug transporter, P-glycoprotein (Pgp), which is a transmembrane transport protein capable of drug expulsion and maintenance of tolerable intracellular levels of certain cytotoxic drugs (2, 3). Pgp belongs to the ATP binding cassette (ABC) family of transporter molecules (4, 5), which also includes the MDR-associated protein (MRP) (6–8). Another transporter, the lung resistance related protein (LRP) (9), has been identified as the major component of certain nucleoprotein particles (vaults) which possibly translocate cytotoxic drugs from nuclei to cytoplasmic vesicles, which in turn are emptied at the cell surface (10). Several clinically important anticancer drugs may be removed from neoplastic cells by Pgp-mediated transport, despite the diversity in chemical structures and mechanisms of action (11, 12). This ability is apparently reflected in a negative correlation between Pgp expression and chemosensitivity or survival in leukemias (13, 14), lymphomas (15), osteogenic sarcoma (16), small-cell lung cancer (17), breast cancer (18) and paediatric solid tumours (19, 20).

Mark Baekelandt from the Norwegian Radium Hospital presented data on the prognostic significance of Pgp expression in ovarian carcinoma. By means of immunohistochemistry, tumour specimens from 73 patients with untreated stage III disease were analysed for Pgp expression. Pgp correlated with poor response to epirubicin and cisplatin combination chemotherapy. Patients with Pgp-positive tumours (47%) differed significantly from patients with Pgp negative tumours with respect to both disease-free survival ($p = 0.001$, chi-square) and corrected survival ($p = 0.0075$, chi-square). In multivariate analysis, Pgp turned out to be the most important single prognostic factor in stage III ovarian carcinoma.

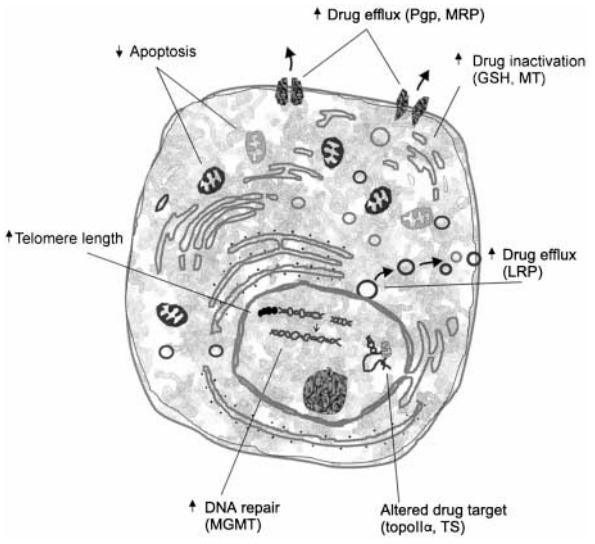

Fig. 1. Schematic drawing of diverse resistance mechanisms, which may be working separately or in concert, in a chemoresistant cell. Pgp = P-glycoprotein, MRP = MDR related protein, LRP = lung resistance related protein, GSH = glutathione, MT = metallothionein, TopoIIα = topoisomerase II-α, TS = thymidylate synthase, MGMT = O6-methylguanine-DNA-methyltransferase.

Since evaluation of Pgp expression may fail because of inaccessible tumour specimens, it would be beneficial to monitor the expression of Pgp in vivo. Astrid Gruber et al. from Karolinska Hospital, Stockholm, presented a poster which reported on evaluation of the MDR phenotype in leukemic cells from patients with acute myelogenous leukemia (AML) using the radioactive isotope $^{99}Tc^{m}$-methoxyisobutylisonitrile (MIBI), which appears to be a substrate of Pgp (21). The intracellular accumulation of $^{99}Tc^{m}$-MIBI was measured with a gammacounter. In Pgp-positive cells, MIBI accumulated to a lesser extent than in Pgp-negative cells ($p = 0.01$, *t*-test), and exposure to Pgp inhibitors increased MIBI accumulation to a higher degree in Pgp-positive than in Ppg-negative cells ($p = 0.01$, *t*-test). Thus, in agreement with studies on renal cell carcinoma and gliomas (22, 23), MIBI scintigraphy provided a valid measure of Pgp function in leukemia cells. However, more studies are warranted to prove the feasibility of MIBI scintigraphy in monitoring Pgp expression and function in vivo, which could be an attractive diagnostic option in chemoresistant cancers.

Drug transport by Pgp is energy dependent and directly linked to hydrolysis of ATP by Pgp-specific ATPase activity (24). Both anticancer drugs and chemosensitizers exhibit a bell-shaped dose–response relationship for Pgp ATPase activity, suggesting the involvement of both activating and inhibiting binding sites (25). Thomas Litman from the University Hospital Herlev, Copenhagen, demonstrated the diversity in substrate interaction with Pgp in isolated membranes of Chinese hamster ovary cells. In these membranes, where Pgp constitutes 30% of the total protein content, it was possible to demonstrate the complex binding behaviour of Pgp. Two distinct binding sites were found to be responsible for activating and inhibiting Pgp ATPase, respectively, which is in agreement with the recent finding by Dey et al. (26) of at least two non-identical substrate interaction sites in Pgp. The high affinity chemosensitizer PSC 833 down-regulates the enzyme activity strongly. Conversely, verapamil stimulates Pgp ATPase (27), which suggests uncoupling of Pgp-mediated drug efflux from ATP hydrolysis. The opposing interactions with Pgp ATPase demonstrate the complexity of Pgp-mediated drug transport and reflect separate targets for Pgp modulation.

Christian Maare from Herlev University Hospital, Copenhagen, reported on the relationship between Pgp expression and the transport kinetics of daunorubicin and epipodophyllotoxins in Ehrlich ascites tumour cell lines expressing diverse levels of Pgp. Expression of Pgp correlated with cytotoxicity tests and efflux of daunorubicin,

etoposide and teniposide. Verapamil restored the intracellular concentration of daunorubicin. On the other hand, concentrations of the epipodophyllotoxins were only partly restored by verapamil, suggesting an alternate efflux mechanism for these cytotoxics.

Pgp may be co-expressed with other MDR phenotypes, as demonstrated by Peter Buhl Jensen from the Finsen Center, Copenhagen, in H69 small-cell lung cancer cells exposed to etoposide for a great number of passages. Evaluation of resistant H69/VP cells using reverse transcriptase-polymerase chain reaction (RT-PCR), immunoblot analysis, and immunocytochemistry showed that Pgp, MRP, LRP, and drug resistance due to altered topoisomerase II-α (topo II-α) drug target (at-MDR) became co-overexpressed in the same cells. During continuous selection with etoposide at-MDR and LRP emerged after 7 passages, MRP after 12 passages and Pgp after 23 passages. Despite the concomitant expression of 4 resistance factors, the H69/VP cells exhibited up to 5-fold collateral sensitivity to the antimetabolites cytarabine and gemcitabin (28). The results suggest that circumvention of MDR could be achieved using drugs to which the resistant cells show collateral sensitivity.

INHIBITION OF DRUG EFFLUX MECHANISMS

The calcium channel blocker verapamil was the first agent to modify MDR in vitro and in vivo (29), but unfortunately the MDR-modifying activity required concentrations that are associated with severe cardiac toxicity in patients (30, 31). Cyclosporin A (CsA) has also been shown to inhibit Pgp potently in both cell lines (32) and in animal models (33), but simultaneous treatment with cytotoxic agents is associated with severe infection risk (34). Therefore, the non-immunosuppressant cyclosporin, PSC 833, which is 10-fold more potent than CsA with respect to MDR reversal (35, 36), represents one of the most promising resistance-modifying agents presently being investigated.

Currently, PSC 833 is the object for a number of clinical trials in Europe and the United States. Jan Liliemark from Karolinska Hospital, Stockholm, presented data from a phase I/II study with cytarabine (1 g/m^2 q 12 h $\times$ 8, daunorubicin (45 mg/m^2 q 24 h $\times$ 3) and PSC 833 in refractory patients with AML. The dose of PSC 833 was escalated from 2 mg/kg/day to 10 mg/kg/day as continuous infusion days 1–4. Because Pgp inhibition delays hepatic and renal elimination of MDR-susceptible drugs (37), dose escalation of PSC 833 required dose reduction of daunorubicin from 45 mg/m^2/day to 25 mg/m^2/day to avoid excessive bone marrow toxicity. After modification of the criteria for dose-limiting toxicity, the dose of daunorubicin could be escalated back to 45 mg/m^2/day maintaining the PSC 833 dose at 10 mg/kg/day (38). A total of 52 patients were included in the study, 11 patients obtained CR, and 6 patients had remission duration of more than 6 months.

Christer Paul, Huddinge Hospital, Stockholm, presented another study addressing the pharmacokinetics of 10 patients with AML who had been treated with daunorubicin 33 mg/m^2/day in combination with PSC 833 10 mg/kg. The administration of PSC 833 was started 24 h after that of daunorubicin. The plasma concentration of daunorubicin increased 50–100% upon co-administration with PSC 833, and the cell/plasma concentration ratio increased 40% in 7 Pgp-positive patients but remained unchanged in 3 patients with Pgp-negative status. Thus, PSC 833 appears to restore the accumulation of daunorubicin in the Pgp-positive leukemic blasts in vivo.

Although Pgp contributes to the renal and hepatic elimination of exogenous toxins, and is part of the blood-brain barrier (39, 40), the physiological role of Pgp is not fully understood. Two recent reports have shown that Pgp may also play a part in the transport of growth-stimulating cytokines IL-2 and IL-4 (41, 42). Gustav Lehne from The National Hospital, Oslo, presented a study addressing the potential growth-regulating role of Pgp in leukemia. The MDR phenotypes of KG1a and K562 leukemia cell lines, which have Pgp overexpression in common, respond to Pgp inhibition by PSC 833 (Novartis), 280–446 (Novartis), or LY335979 (Lilly) with growth reduction in a dose-dependent manner and emergence of apoptosis. Leukemia cells devoid of Pgp were not affected. The apparent selective cytotoxicity should be considered during the ongoing clinical trials of PSC 833 and suggests a potential role for monotherapy with PSC 833 and related agents in future therapeutics of chemoresistant cancer.

MECHANISMS OF DRUG DETOXIFICATION

Once cytotoxic drugs have entered the intracellular compartment of tumour cells, a number of mechanisms permit detoxification. Among these are glutathione (GSH) and metallothioneins (MTs), which contain electron-rich sulfhydyl groups capable of inactivating alkylating agents and cisplatin (43, 44). A group of cytosolic enzymes termed glutathione-S-transferases (GSTs) are responsible for the conjugation of these drugs with GSH. This detoxification mechanism may require vesicle-mediated transport of GSH-drug conjugates by a poorly understood ATP-dependent GS-X pump. The GSH economy of cancer cells is only partly understood, but depletion of GSH makes cells more vulnerable to the drugs involved.

MTs are low molecular weight intracellular proteins characterized by high cystein content and affinity for binding heavy metals. These proteins are mainly located in parenchymal tissues like liver, gut and kidneys, where they play a role in the detoxification of certain heavy metals and in the regulation of normal zinc and copper metabolism. Hans Erik Rugstad from The National Hos-

pital, Oslo, reported on studies addressing the possible role of MT in resistance to cytotoxic agents, irradiation and tumour necrosis factor (45, 46). Cell lines exposed to cadmium increase the concentration of cytosolic MT. Such cells are resistant also to alkylating drugs like melfalan and chlorambucil, which were shown to bind to MT by a radiotracing technique. MT also confers resistance to ionizing radiation, probably by neutralizing oxygen radicals. Determination of MT in cancer specimens may provide the clinician with important prognostic information, as MT expression has been shown to be a marker of poor prognosis in breast cancer (47), esophageal cancer (48) and possibly non-seminomatous germ cell tumours (49).

DNA REPAIR AND RESISTANCE TO ALKYLATING AGENTS

Alkylating agents induce lethal DNA damage by forming covalent bonds with nucleophilic sites in the DNA. The N7 nitrogen and O6 oxygen atoms of guanine are probably the main targets for alkylation of DNA. Repair of DNA adducts represents one of the main mechanisms of cellular protection, and one important DNA repair enzyme is O6-methylguanine-DNA-methyltransferase (MGMT), which may remove alkyl adducts from the O6-atom of the guanine base (50). A striking correlation between MGMT activity and resistance to nitrosoureas has been demonstrated both in vitro and in xenograft models (51, 52). However, alkyl transferase activity may vary considerably within a particular cell or tissue type, which might challenge the reproducibility of the analysis in clinical specimens (53–56).

Johan Hansson from the Karolinska Hospital, Stockholm, Sweden, presented data from studies on MGMT expression in biopsies from human melanoma metastases using biochemical activity assays, RT-PCR techniques, immunohistochemistry and immunocytochemistry (57, 58). Biopsies from both cutaneous and lymph node metastases were analysed, and a wide variation in MGMT expression was found in different biopsies, in some cases even when biopsies were obtained from the same patient. Biopsies from patients resistant to the methylating agent dacarbazine had higher average MGMT activities than those from patients with sensitive tumours, although the difference was not statistically significant. Because the alkyltransferase inhibitor O6-benzylguanine potentiates the toxicity of nitrosoureas in xenotransplant models of human tumours (59, 60), MGMT assays may provide valuable guidance concerning individual treatment strategies for circumventing resistance to alkylating agents.

APOPTOSIS

Apoptosis (programmed cell death) is an active regulatory process of controlled autodigestion of the cell which is crucial for tissue homeostasis ensuing that the rate of cell proliferation is offset by a proportionate rate of cell loss (61). The death process is a complex chain of reactions regulated by certain genes, including the oncogene *BCL-2* and the tumour suppressor gene *P53*, which participate in suppression and progression of the apoptotic pathway, respectively. Cells that undergo apoptosis are characterized by shrinkage, plasma membrane blebbing, nuclear fragmentation, chromatin condensation and finally disintegration into apoptotic bodies. In contrast to necrotic cell death apoptotic cells are immediately phagocytozed by neighbouring cells without evoking an inflammatory response. Overexpression of bcl-2 protein, which is common in malignant lymphomas, prolongs cell survival by avoidance of apoptosis (62). Resistance to apoptosis is closely linked to drug resistance because a number of chemotherapeutic agents act by inducing apoptosis (63, 64). Subsequent to drug-target interaction, cytochrome C is released from mitochondria into the cytosol to activate certain cysteine proteases termed caspases, which promote apoptosis by proteolytic degradation of cellular components and activation of endonucleases leading to degradation of chromosomal DNA into oligonucleosomal fragments (65). Thus, *P53* mutations, up-regulation of *BCL-2* and defects in caspase activation may confer MDR in malignant cells (66–69).

The guest speaker Caroline Dive from the School of Biological Sciences, Manchester University, presented data that links apoptosis regulation with the acquisition of drug resistance in low-grade follicular B-cell lymphoma. Despite the introduction of new drug regimens the survival of this malignancy has not improved for decades, and development of MDR is still inevitable in these patients. An in vitro cell-free system was developed to mimic the survival niche of lymphoma cells within a germinal centre (70). The survival signals working in the germinal centre are CD40 receptor ligation, interleukin-4, vascular cell adhesion molecule-1, and an interaction with extracellular matrix through intergrin receptors. Burkitt lymphoma cell lines were used to show that germinal centre signals suppress chlorambucil-induced apoptosis and thus provide drug resistance to these cells. Overexpression of the oncoprotein bcl-2 did not prevent apoptosis induction by chlorambucil, but the germinal centre survival signals did. These survival signals also led to upregulation of anti-apoptotic bcl-xL protein and a clonogenic survival, which suggest one possible mechanism for acquired resistance in low-grade B-cell lymphomas.

Ellen Friche from the Finsen Center, Copenhagen, presented data on Pgp expression and induction of apoptosis in human CEM cells derived from acute T-lymphoblastic leukemia and three MDR variants with different grades of resistance to vinblastine. The sensitive parental cells accumulated in the G2/M cell-cycle phase in response to vin-

cristine exposure for 24 h, and were extensively apoptotic as demonstrated by DNA gelelectrophoresis and morphological examination. On the other hand, vincristine was unable to influence the cell kinetics or to induce apoptosis in the most vinblastine-resistant cell line, unless cultured together with PSC 833. The induction of apoptosis was coincident with the appearance of a phosphorylated bcl-2 protein in immunoblot analysis. Thus, despite development of resistance to vinblastine and Pgp overexpression, the apoptotic pathway was intact. Jens Eriksen from University Hospital, Herlev, Copenhagen, arrived at the same conclusion for leukemic K562 cells with diverse resistance patterns. During single agent multistep selection with various anticancer drugs the apoptosis regulation was highly conserved, although selection with vincristine conferred 2.5-fold resistance to apoptosis induced by cisplatin and staurosporine.

Resistance to one type of apoptosis does not preclude apoptosis induction by alternate pathways. There are several options for apoptosis and Stein Ove Døskeland from the University of Bergen addressed the influence of activation pathways on the apoptotic phenotype in his lecture. Difference in apoptotic morphology seems to be dependent on activation pathway, and Døskeland demonstrated four distinct phenotypes secondary to activation by cAMP, anti-Fas antibody, okadaic acid, and caliculin A (71). Interestingly, the latter two substances, which are phosphatase inhibitors, induce little or no DNA fragmentation at all.

Døskeland also demonstrated that overexpression of bcl-2 in leukemic cells can confer 150-fold resistance to the cytotoxic agent daunorubicin. Bcl-2 blocks apoptosis induction by Fas and cAMP. Marine toxins such as microcystin, which are potent phosphatase inhibitors, may override the antiapoptotic effect of bcl-2. These agents induce very rapid death within minutes (72), but the death pathway may be blocked by kinase inhibitors. An alternative approach to circumvent bcl-2-mediated drug resistance is *BCL-2* antisense therapy, which recently was shown to sensitize xenotransplanted human malignant melanoma to dacarbazine (73).

The p53 protein is a transcriptional activator of specific genes necessary for growth inhibition and induction of apoptosis (74). Functional p53 is required for G_1 cell cycle arrest in response to DNA damage. Mutations of the *P53* gene are frequently seen in human cancers, and allow uncontrolled growth of damaged cells. The defective cellular response after DNA damage may lead to tumour progression and chemoresistance (75, 76).

In breast cancer, at least 25–30% of the tumours harbour *P53* mutations. Mutant p53 protein usually has a prolonged half-life and, therefore, it can be stained immunohistochemically. However, some of the mutated genes cannot be detected by this method because of missense mutations and stop codons which destroy large parts of the gene and corresponding proteins. Per Eystein Lønning from Haukeland Hospital, Bergen, reported a study on de novo resistance to doxorubicin in breast cancer patients with specific *P53* mutations (77). A total of 63 patients with locally advanced breast cancer were treated with weekly doxorubicin 14 mg/m^2 up to 16 weeks. The *P53* status was evaluated in biopsies taken before and after the treatment. Mutations of the *P53* gene occurred in 18 patients (29%) as evaluated by constant denaturant gel electrophoresis or direct cDNA sequencing. The survival of stage III patients with *P53* mutations affecting the L2/L3 domains of the p53 protein was inferior to the survival of comparable patients without mutations or mutations elsewhere ($p = 0.0093$, log-rank).

Therapeutic approaches to circumvent chemoresistance caused by *P53* mutations are awaited with considerable interest. It is still not known whether high-dose therapy or treatment with taxanes, which cause apoptosis independently of p53, may improve the prognosis in patients with mutant *P53*. Preliminary experiments with gene therapeutic replacement of mutant *P53* have already been successful in sensitizing tumour cells to chemotherapy in vitro and in vivo (78, 79).

TELOMERASE

A new possible target for antineoplastic treatment is the ribonucleoprotein enzyme, telomerase, which synthesizes telomeric repeats and consequently counteracts telomere shortening. Telomeres are functional units of proteins and repetitive DNA sequences (80). They protect chromosomes from loss of DNA, but lose terminal sequences themselves with every cell division. Thus, telomere length decreases with ageing, which leads to limited replicative capacity, senescence and eventually death. Increased telomerase activity may, therefore, be regarded as a potential drug resistance mechanism. Astrid Gruber from Karolinska Hospital, Stockholm, presented a study of the clinical relevance of telomerase activity in AML. Telomerase activity was measured in 95 leukemic cell samples from 66 patients using a telomerase PCR ELISA kit, and the activity was increased compared to peripheral blood mononuclear cells from healthy adults in 82 samples (86%). Moreover, the telomerase activity increased at relapse or progression ($p = 0.003$, t-test) compared to disease onset, and correlated positively with known adverse prognostic factors such as CD34 expression and chromosome aberration. Upregulation of telomerase activity occurs frequently in AML, but it remains to be shown that increased telomerase activity is an independent marker of poor prognosis and drug resistance. Recently, it has been demonstrated that telomerase activity in human breast cancer is associated with a more aggressive phenotype in patients (81).

DRUG SENSITIVITY TESTS

Valid methods for predicting clinical response to anticancer drugs would provide a means by which to avoid chemoresistance by drug selection based on activity in tumour specimens ex vivo. Several attempts have been made with various techniques utilizing the activity of specific enzymes of viable cells to convert certain reagents to chromophores or fluorophores (82–84). One of the most successful methods is the fluorometric microculture cytotoxic assay (FMCA), which measures fluorescence generated from hydrolysis of fluorescein diacaetate to fluorescein by living cells. Rolf Larsson et al. from the University Hospital in Uppsala have studied with FMCA assay the effects of 18 anticancer drugs on 4 haematological and 9 solid tumour types from more than 1700 clinical specimens. High in vitro activity was observed in haematological tumours for most of the drugs. The drug activity in solid tumours was generally lower except in paediatric tumours and in small-cell lung cancer. In vitro response rates were compared with clinical response rates obtained from the literature. The in vitro sensitivity correlated with the retrospective clinical data for a number of drugs, but the correlation needs to be confirmed in prospective studies.

DRUG TARGET REGULATION

DNA topoisomerases are nuclear enzymes that play essential roles in DNA metabolism. The nuclear enzyme topo II, which cuts and passes double-stranded DNA during replication, is the primary target for many commonly used anticancer drugs such as etoposide and teniposide. Tumours may have different ways of modulating the level, structure and activity of topo II in such a way that their sensitivity to the cytostatic drug becomes reduced. Since topo II actively participates in the formation of critical DNA lesions, decreased enzyme content has been associated with a more drug-resistant phenotype (85–87). Jesper Kreisholt et al. from the Finsen Center, Copenhagen, presented a poster that demonstrated frequent decrease in immunohistochemical expression of topo IIα in biopsies from patients with small-cell lung cancer (SCLC) after treatment with topo II drugs. These findings imply that treatment of SCLC with topo II drugs frequently results in selection of the altered topoisomerase MDR (at-MDR) phenotype. They also found that the topo II expression was significantly lower in untreated non-small-cell lung cancer (NSCLC) than in untreated SCLC which might explain the intrinsic MDR associated with NSCLC.

Thymidylate synthase (TS) is the target enzyme for 5-fluorouracil (5FU), which requires conversion to the active drug, fluorodeoxyuridylate (FdUMP) to form an inhibitory complex with TS and folate co-factors. Further conversion of dUMP into thymidylate is blocked by the stability of the fluorine carbone bond on FdUMP, and consequently necessary thymidylate constituents of DNA are deleted. Experimental studies have shown that increase of TS concentration or decrease of its ability to bind FdUMP may induce resistance to 5FU (88, 89).

Bengt Gustavsson from Sahlgrenska University Hospital discussed the importance of quantifying TS in tumour specimens to understand the mechanisms of 5FU resistance. Using sensitive methods, quantitation of TS can be performed in very small tumour biopsy samples (90, 91). In clinical specimens there is a very wide range of TS concentrations in solid tumours. Several studies have shown that TS concentrations are higher in poorly differentiated tumours than in well-differentiated tumours and high TS levels correlate with poor prognosis. Furthermore, the concentrations of TS are higher in tumour tissue than in normal liver tissue (92). Decreased levels of reduced folates may contribute to insufficient TS inhibition (93). On the other hand, the addition of high-dose leucovorin, a prodrug of tetrahydrofolates, is associated with increased TS inhibition (94). The response to 5FU is also improved when short-time infusions are replaced by bolus injections, which result in significantly increased bioavailability and serum peak concentrations of the drug (95).

CONCLUSION

The First Nordic Conference on Chemoresistance in Cancer reflected an immense width in the research currently being conducted by the Nordic academies in the field of chemoresistance. A range of mechanisms which contribute to this challenging obstacle to successful chemotherapy were elucidated, and the complexity of this issue was emphasized. However, more studies on development of reliable diagnoses of the different resistance factors are warranted. High quality diagnostic procedures will not only unveil the clinical significance of the different mechanisms of chemoresistance, they will also provide guidance to clinical trials addressing circumvention of chemoresistance with novel therapeutic approaches.

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