Review

Multidrug resistance: A solvable problem?

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Summary

The results of chemotherapy of pancreatic cancer have been disappointing thusfar. Pancreatic tumors are primary resistant to available drugs. Why that is, is as hard to define as why normal tissues are resistant. Meaningful studies on drug resistance of pancreatic tumors can only be expected after a highly effective drug has been found. While waiting for this magic bullet to arrive, I shall review our knowledge of drug pumps used by other cancer cells to extrude drug from the cells as a method to escape initially effective chemotherapy.

Key words: drugresistance, MRP, P-glycoprotein

Introduction

The pancreas is not popular with molecular biologists. It teems with enzymes that degrade the molecules that molecular biologists like, nucleic acids and proteins. Even though these enzymes are present as pro-enzymes, the inactive precursor proteins are readily activated during standard isolation procedures. I still remember the time when it was impossible to isolate high-molecular weight RNA from pancreas at all.

Pancreas is also not popular with biochemists working on drug resistance. This is not for lack of material. Pancreatic cell-lines are available that are representative of the properties of pancreatic epithelial cells. They should be equally suitable for work on drug resistance as HeLa cells or the popular lung carcinoma cell-lines. The reason that pancreatic cell-lines are unpopular for drug resistance studies, is that pancreatic carcinoma is not susceptible to drug treatment. A recent comprehensive book on pancreatic carcinoma does not even have a chapter on chemotherapy [1]. Drugs are only used in an adjuvant setting and with marginal (and controversial) results.

Why do biochemists not work on tumors that are already resistant at the outset? This is not because the problem is uninteresting, but because it is not accessible. Primary resistance usually means that the tissue of origin is relatively well-protected against drugs and that the tumor has not lost this protection during the multi-step process of carcinogenesis. The protection mechanisms of normal tissues are biochemically complex and are only slowly being dissected. More importantly, if no drug is available that shows preferential killing of pancreatic cancer cells, it is not very useful to know why these cells are resistant. Even if one would find methods to make them less resistant it is very unlikely that these methods would not also increase the sensitivity of normal cells to drugs. Hence, biochemists do not work on drug resistance in pancreatic cancer cells as long as clinicians and the pharmaceutical industry have not managed to find a drug that is effective against pancreatic cancer. Once such a drug is available, patients will relapse, because of drug resistance, and then biochemists shall step in to search for mechanisms of drug resistance and ways to circumvent them.

This is how biochemists usually work: they take a tumor that is sensitive to chemotherapy. They derive cell lines in vitro and select resistant mutants. With a lot of hard work the resistance mechanism or mechanisms may sometimes be found and methods developed to recognize the proteins involved in resistance in patient samples. It is then possible to test whether the mechanisms play a role in patients that relapse after initial response to the drug.

While we wait for drug companies and clinicians to find a new magic bullet, let me briefly summarize how cells defend themselves against the available bullets. My own interest is in multidrug resistance and I shall concentrate on this topic here.

Multidrug resistance; how are we doing?

In the past 20 years, we have made substantial progress in understanding MDR and in generating the means to tackle it. The classical definition of MDR emphasizes the diversity of drug types and intra-cellular drug targets involved. We now know that two types of resistance fall under this definition: 1. Resistance due to drug pumps [2]. This type of resistance mainly affects large amphipathic drugs, such as anthracyclines, epipodophyllotoxins, and vinca alkaloids, which enter the cell rather sluggishly by passive diffusion. As entry can not be changed, all living organisms, from bacteria to humans, have developed drug pumps, usually located in the plasma membrane, that extrude these drugs from the cell as soon as they enter. 2. Resistance due to interference with apoptosis. Many anti-cancer drugs appear to act by inducing apoptosis in the recipient cell. Decreased apoptosis can therefore lead to drug...
resistance, at least in cultured cells. Whether it does so in patients, remains to be seen.

Biochemical research has uncovered three types of drug pumps that may play a role in MDR. The most intensely studied is the P-glycoprotein encoded by the MDR1 gene in humans. A variety of studies have shown that this protein acts as a drug pump in the plasma membrane of tumor cells and that it can recognize an astonishing range of cytotoxic molecules and remove them from the cell. We have generated mice unable to make any drug-transporting P-glycoprotein. These are completely healthy and only hypersensitive to drugs normally transported by P-glycoprotein [3]. This confirms that the main role of this protein is in defence of the body against xenotoxins.

Whether the increased levels of P-glycoprotein found in some drug-resistant tumors actually contribute to resistance is still a controversial issue. I find it a priori unlikely that tumor cells would forego a perfectly fine mechanism of resistance, if available. Tumors are genetically heterogeneous and highly opportunistic. Whatever mutation is likely to allow them to continue multiplying will probably be selected for.

Compelling evidence for this type of selection has recently come from work of Fojo's group at the NCI (USA). Mickley et al. [4] analysed the nature of the overexpressed MDR1 allele(s) in myeloma cells from patients in which the two alleles present could be distinguished by a polymorphism. In each case in which MDR1 was overexpressed, only a single allele was found overexpressed. The authors modestly conclude that "the results in these patient samples support the idea that MDR1 played a role in drug resistance these tumors in the course of therapy". I find this persuasive evidence that MDR1 does play a role. There is no reasonable other mechanism that would consistently select for overexpression of a single allele. Ongoing clinical trials in which chemotherapy is combined with an effective inhibitor of P-glycoprotein, such as the cyclosporin A analogue, PSC 833, should eventually settle whether P-glycoprotein significantly contributes to MDR of those tumors in which it is overexpressed.

The multidrug resistance (associated) protein, MRP1

A second class of transporters potentially involved in MDR, is known as glutathione-drug-conjugate pumps, or GS-X pumps. The prototype is the Multidrug Resistance (associated) Protein MRP1, discovered by Cole and Deeley [5]. MRP1 can transport a large range of drugs conjugated to negatively charged hydrophilic ligands such as glutathione, glucuronic acid and sulphate. Indirect evidence strongly suggests that this class of pumps can also transport complexes of glutathione with cisplatin, with arsenite or with antimonite. MRP1 is also able to confer resistance to drugs that are not known to be conjugated to any acidic ligand, such as doxorubicin or vinca alkaloids. Resistance requires the presence of glutathione, however, and all available evidence indicates that MRP1 can co-transport drug with glutathione and even (at low rate) glutathione alone (R. Evers and P. Borst, unpublished). Not all hydrophobic anti-cancer drugs are efficiently transported by MRP1. Paclitaxel, for instance, is a good substrate for P-glycoprotein but a poor one for MRP1. The MRP1 gene is expressed in nearly all human tissues and MRP1 is found in many tumors. Although it is likely to contribute to clinical MDR, the correlations between MRP1 levels and resistance are not strong. Unfortunately there is no highly effective non-toxic inhibitor available for MRP1 yet. This is a prerequisite for the dissection of its potential clinical role.

We have studied the physiological function of MRP1 by generating knock-out mice [6,7]. The mice are fine, moderately hypersensitive to etoposide, but not cisplatin. Although tissue distribution and pharmacokinetics of etoposide were unaltered in the KO mice [6], more detailed analysis [7] has identified three tissues that are especially affected:

- The oropharyngeal mucosa is destroyed, apparently because the epithelial stem cells are protected by high levels of MRP1.
- Spermatogenesis is abrogated in the seminiferous tubules of the testis, apparently because the MRP1 normally present in the basolateral membrane of the Sertoli cells is an important part of the blood-testis barrier to drugs.
- The mice suffer from drug-induced polyuria and haemoconcentration resulting apparently from damage of the urinary collecting tubules, another cell type that prominently stains with anti-MRP1 antibodies.

Other MRP's

MRP1 is part of a family of transporters that now number 7 members, MRP1-7. The best characterized member thusfar is MRP2, also known as the canalicular Multispecific Organic Anion Transporter (cMOAT). The major location of this transporter in the body is in the canalicular membrane of the hepatocyte where it is responsible for the excretion of conjugated bilirubin and other organic anions. There is a host of other drugtransporters (or putative drug transporters) that might also contribute to MDR [reviewed in ref. 8]. Of the other MRP family members, cMOAT (MRP2) is best characterized.

In experiments with cells transfected with an MRP2 construct, transport of vinblastine and resistance to mitoxanthrone, but not to cisplatin (M. Kool and P. B., unpublished), have been shown. So, in principle, overexpression of MRP2 could contribute to MDR, in human tumors, but whether this occurs in practice remains to be determined.

Less is known about MRP3-6 and nothing yet about MRP7. In experiments with cells transfected with an MRP2 construct, transport of vinblastine and resistance to mitoxanthrone, but not to cisplatin (M. Kool and P. B., unpublished), have been shown. So, in principle, overexpression of MRP2 could contribute to MDR, in human tumors, but whether this occurs in practice remains to be determined.

Less is known about MRP3-6 and nothing yet about MRP7. We have spent considerable time on analysing the transport activities of MRP3. By transfection studies we have shown that overexpression of MRP3 can render cells resistant to etoposide and methotrexate (M. Kool and P. B., unpublished). It is therefore probably also an organic anion pump. This pump is present in the small intrahepatic bile ducts, in the adrenal cortex (mainly in the zona fasciculata), in colon and in the proximal tubuli of the kidney (M. Kool and P. Borst, unpublished). The physiological function of MRP3 is not yet clear; we found low transport of estradiol...
through cells overexpressing MRP3 and the protein may therefore transport steroid conjugates. Whether it can contribute to MDR in patients remains to be seen.

MRP6 is adjacent to MRP1 in chromosome 16 and its overexpression in MDR lines can be fully explained by co-amplification of MRP1 and MRP6. The same explanation holds for the Anthracycline Resistance Associated (ARA) gene found overexpressed by others in resistant leukemia cells. We have shown that the putative ARA protein is almost identical to the C-terminal part of MRP6 and that partial co-amplification of MRP6 with MRP1 can account for the ARA RNA found in resistant cells [9].

MDR-related proteins in pancreatic tumors

No systematic studies have been done on the level of MDR-related proteins in pancreatic cancer. In a survey of tumors, >70% stained strongly positive for P-glycoprotein and staining was inversely correlated with biological aggressiveness [10]. Serially transplanted human cell lines were mainly sensitive to cisplatin and mitomycin, but not to doxorubicin [11]. Analysis of pancreatic tumor cell lines showed the presence of MRP1, but no P-glycoprotein [12]. A cell line selected for high doxorubicin resistance in vitro, showed decreased drug accumulation not attributable to either P-glycoprotein or MRP1 overexpression [13]. The authors speculate that increased drug exocytosis might cause resistance, but this interpretation remains to be substantiated. There is no example yet of increased exocytosis as a proven mechanism for MDR.

Outlook

Is MDR in cancer cells a solvable problem? Yes and no, is my answer to this question. Yes, because we can foresee the day that we can detect and understand all drug resistance mechanisms in cancer cells. The human genome project will provide us with an inventory of all human genes. Studies on drug-resistant cells will provide insight in possible resistance mechanisms. Our ability to modify the mouse genome, provides a powerful tool to unravel the function of genes in the context of a living mammal, which resembles us sufficiently to allow extrapolation to humans. So yes, in 20 years or so we shall understand MDR and drug resistance in cancer. We may also expect with cautious optimism that we shall be able to use this knowledge to improve treatment of cancer patients.

My other answer is no, multidrug resistance will never be completely solved, even if we understand all mechanisms involved completely, for the simple reason that cancer cells are very similar to normal cells. Mutations in five to seven genes suffice to turn a normal cell into a cancer cell. Since human cells have about 100,000 genes, the mutations that cause cancer affect only 0.01% of all genes. Obviously cancer cells may destroy their host, like invading microorganisms, but they are still human cells with enzymatic reactions that are virtually identical to those of the normal tissue from which the tumor was derived. Hence, all the chemotherapeutic agents that are so effective in exploiting the differences in metabolism between micro-organisms and human cells are useless for tumor cells. Worse, the defence mechanisms employed by tumor cells against anti-cancer drugs are the very same that normal host cells use for defence against drugs and other xenobiotics. Even if we find suitable inhibitors to deal with any form of MDR, - and this is a big if -, even then we shall only be able to level the playing field, i.e. remove the defensive system both in tumor cells and in normal cells. It will then still be necessary to find drugs with sufficient specificity for the tumor to kill all tumor cells without substantial damage to the host. We shall advance step by step on this road, but I do not expect that we are going to see the end in our lifetime.

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References


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