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Drug Resistance and Cancer Therapy

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Many patients undergo chemotherapy treatment and achieve a complete remission. By all means of assessing cancer burden in the body, these individuals appear to be cured. Yet ultimately, many patients are doomed to relapse and death due to cancer. There are many mechanisms by which cancer drugs may be ineffective for a given individual. Resistance may be due to pharmacokinetic, cytokinetic, cellular and molecular mechanisms. As medical oncology becomes more sophisticated, our ability to identify and modulate these various levels of drug resistance will improve survival and cure rates for patients with cancer.

Pharmacokinetics plays an important role in chemotherapy failure. Drugs may not be activated or may be rapidly inactivated or excreted by organs such as the liver and kidney. Tumor cells may be present in drug-inaccessible "sanctuary sites", such as the CNS, eyes, and testes, leading to relapse in these organs. Tumors with poor blood supply may receive inadequate drug levels to effect complete killing of all cells. Increased interstitial pressure within tumors, or the presence of excessive extracellular matrix may limit drug penetration to the site of active tumor cells. Inadequate drug dosing or inappropriate drug scheduling may be important. Route of administration may be critical, as some oral drugs vary in bioavailability. The problems of effective pharmacokinetic delivery and activity of anticancer drugs has been the traditional concept of drug resistance, and has been the focus of research by pharmaceutical companies intent on producing effective anticancer agents for years.

Inherent drug resistance -The Goldie-Coldman hypothesis states that 1 in 10^6 cancer cells is inherently resistant to a given chemotherapeutic drug, or class of drugs. The Goldie-Coldman hypothesis is based on an understanding of tumor cell population kinetics and rates of mutations inherent in mammalian cells. Damage to DNA is a constantly occurring fact of life for all cells. DNA repair is not perfect. In some cases, base mismatch can occur as the result of faulty excision repair of bases damaged by UV light or other genetic toxins. DNA replication is not perfect. DNA polymerase is a highly efficient enzyme, but base mis-incorporation occurs at a predictable rate of 10^6 bases. Fortunately, most of these point mutations will be functionally silent, but some will result in serious consequences to the cell. Because these sources of mutation are inherent in living cells, there is an inherent mutation rate. One consequence of this mutation rate is the development of resistance to drugs before any exposure to the drugs takes place. This resistance is thus a form of inherent drug resistance.

Acquired drug resistance -In addition to this inherent drug resistance, drug resistance can be acquired by cellular or organ contact with sublethal concentrations of certain chemicals, pollutants, and drugs. Exposure to toxins results in the upregulation of cellular defense mechanisms, which may result in cross-resistance to the toxic drugs we administer as therapy for cancer. These chemicals in fact result in an induced or acquired drug resistance in the organism. However, exposure to sub-curative levels of chemotherapeutic drug

themselves results in what is classically called acquired drug resistance.

Molecular mechanisms of drug resistance - Examples of cellular resistance mechanisms include the cell's ability to exclude the drug from cell membrane uptake, to repair damage to DNA, and to replace or repair substrates for DNA that have been inhibited by the drugs. The first described phenomenon of pleiotropic drug resistance to be identified involved inherently resistant or acquired resistance to hydrophobic chemotherapeutics containing complex chemical ring structures.

MDR -This pleiotropic resistance mechanism involves amplification one of a group of genes called the MDR ("Multiple Drug Resistance") genes, which may be amplified after repeated exposures to drugs. The MDR genes are believed to confer a detoxifying ability to normal cells in contact with environmental xenobiotic toxins. Because of the importance of protecting cells against these xenobiotic toxic insults, nature has provided a high level of redundancy in these protective systems. Essentially, the products of MDR-type genes are cell membrane ATP-dependent pumps, which immediately remove certain classes of toxins from cells. There are 3 known genes in the MDR class identified in the dog, plus similar but less well evaluated genes in the MRP and LRP families. Cells of the colon, kidney, small intestine, adrenal gland, and liver contain multiple copies of an MDR gene before exposure to drug. The gene is also expressed in bone marrow stem cells and the capillary endothelial cells of the brain, contributing to the blood-brain barrier. All cells that express MDR at high baseline levels, and consequently the cancers that arise from these cells, are inherently resistant to certain classes of toxins and anticancer drugs. The phenomenon of pleiotropic drug resistance is a great problem, in that cross-reactive resistance to many of the commonly used chemotherapeutic agents allows for relapse of previously controlled cancers. An energy-dependent drug efflux pump (the p-glycoprotein pump) causes rapid export of vincristine, vinblastine, teniposide, etoposide, doxorubicin, epirubicin, mitoxantrone, mithramycin, daunomycin, actinomycin and the taxanes. These drugs share the common characteristics of being lipophilic, 300 to 900 daltons in molecular weight, negatively charged, and capable of passive diffusion into cells. Calcium channel blockers, among other drugs, can overcome this resistance in cell culture systems but have thus far proven too toxic for clinical use. New genes associated with the MDR phenotype continue to be identified.

Sulfation and drug resistance -Resistance to the alkylating agents and platinum agents is mediated in part by pathways associated with glutathione metabolism. Glutathione is the most abundant non-protein thiol in cells and is the universal protectant against electrophilic attack directed against DNA and proteins of cells. All of the alkylating agents are electrophiles that damage DNA by forming covalent bonds with DNA, particularly the guanine base. High intracellular levels of glutathione allow for conjugation and inactivation of the reactive alkylating moiety. The enzyme glutathione S-transferase (GST) catalyzes the conjugation of the drugs with glutathione, so cells with very high inherent levels of glutathione or high GST activity levels tend to be resistant to alkylating agents. There are 4 classes of glutathione S-transferases, and the different isoforms of this enzyme apparently catalyze reactions with different alkylating agents. Hence, cross-resistance among drugs in this class may not be absolute. High levels of glutathione or metallothionine are also involved in resistance to platinum agents.

DNA repair and drug resistance - DNA repair enzymes are also important in drug resistance. In the case of drugs such as the alkylators such as nitrosoureas, lethal DNA cross-links are formed between the individual DNA strands. Increased activities of enzymes that cleave these covalent bonds from the DNA allow for repair before a functional effect can be seen. The DNA repair enzyme for BCNU and CCNU is 06 alkyl guanine-DNA alkyl transferase (AGT). When AGT levels are high, nitrosoureas are ineffective. The level of AGT activity can be depleted because the act of repairing the DNA lesion inactivates the enzyme. Thus, competitive substrates for AGT activity can allow nitrosoureas to become fully active against DNA. This process of DNA repair is termed a "suicide repair" because the enzyme doing the repair is permanently inactivated. One agent has been developed to completely deplete AGT activity in tumor cells. This compound is called 06-benzylguanine, and it has been used successfully to enhance sensitivity to BCNU in human trials. Many other similar specific adduct repair pathways have been discovered, and may prove useful as therapy targets in the future.

Enzyme modifications and drug resistance - For certain classes of drugs, such as the antimetabolites and topoisomerase inhibitors, mutations in the binding site of a drug can result in drug resistance. Resistance to paclitaxel and docetaxel may be due to mutated isoforms of alpha and beta tubulins, which prevent binding of drug to its target site. Similarly, high levels of the target enzyme dihydrofolate reductase may mediate methotrexate resistance, such that the molar concentration of drug achievable intracellularly cannot completely block the enzyme. The desired end product, thymine, is still produced in sufficient supply to allow continued replication of the cancer cell. The antimetabolite 5-fluorouracil blocks the enzyme thymidylate synthase and can be overpowered by the amplification of production of the target enzyme. Mutations in these target enzymes can affect binding affinity of the anti metabolites and confer drug resistance as well. Changes in available pools of dinucleotide reductase enzymes may confer resistance to antimetabolites such as cytosine arabinoside and gemcitabine.

The cell cycle, apoptosis, and drug resistance - Eukaryotic cells live in a challenging and often toxic environment. Cell replication is an intricate and complex process that depends upon having an intact DNA template and appropriate substrates, growth factors, and conditions to support replication. The dominant state of the cell is to be in G0 or G1 phases of the cell cycle. This quiescence is maintained by cell cycle regulatory genes, particularly those in the p53 and Rb pathways. Under the appropriate conditions, cells are allowed to progress through the cell cycle to produce 2 identical daughter cells. If a cell has damaged DNA or inadequate resources to proceed through the cell cycle, it is arrested at cell cycle checkpoints. These checkpoints are associated with a molecular clock; if the cell cannot complete DNA repair or if growth factors and building blocks cannot be produced rapidly enough, the cell undergoes programmed cell death, or apoptosis. Apoptosis is thus an ultimate tumor suppressor mechanism, which prevents the accumulation of genotoxic damage in cells. Unfortunately, one of the most common defects in cancers is the loss of p53 cell cycle regulation. In cases with aberrant function of p53 gene, DNA damage may be induced by drugs and radiation, but the cells simply cannot die in response. Also, defects in the pro-apoptotic Rb pathways, or excessive levels of anti-apoptotic cellular survival factors such as bcl2, allow cells to replicate in the face of DNA damage. Under normal

medical circumstances, cells die as a result of exposure to chemotherapy agents because DNA damage triggers apoptotic cell death. When the cell death pathways are defective, standard genotoxic anticancer strategies such as chemotherapy or radiation therapy may in fact promote increased rates of mutation and decreased cell cycle times. Very fast growing cancers may be sensitive to anticancer drugs initially but may then acquire resistance, as cells with defective apoptotic regulation become the dominant cells in the tumor population.

Summary - All of these mechanisms are being studied avidly with the goal of modifying drug resistance factors. If chemotherapy response of malignant cells can be increased, therapeutic outcomes will improve for human as well as veterinary patients. On the other side of the drug resistance problem, researchers are investigating ways to limit anticancer drug toxicity to tissues such as bone marrow and gastrointestinal epithelium by exploiting drug resistance mechanisms that exist in these cells, or by genetically engineering resistance factors for bone marrow transplant rescue after high dose chemotherapy.