SUICIDE GENE THERAPY CAROLINE J. SPRINGER AND ION NICULESCU-DUVAZ pdf

1: Suicide Gene Therapy : Caroline J. Springer :

Suicide gene therapy has become a major element in anticancer gene therapy and holds great promise for the future. In Suicide Gene Therapy: Methods and Reviews, prominent researchers and clinicians comprehensively detail the theory and practice of this exciting and elegant therapeutic approach.

Springer Gene therapy is defined as a technology aimed at modifying the genetic component of cells for therapeutic benefit. Chemotherapeutic suicide gene therapy approaches are known as gene-directed enzyme prodrug therapy or gene-prodrug activation therapy. Other approaches include replacement gene therapy, antisense strategies and induction of resistance to normal cells. All gene therapy strategies share a common component, which is the need for a selective delivery vehicle or vector with tumor- targeting capabilities. This need has led to the in-depth investigation of viruses as new vectors for gene therapy. The conventional approach to the treatm ent of cancer is cleaved to an active drug that kills not only tum or cells with cytotoxic chem otherapy, either alone or in com bi- but also neighboring non-enzym e expressing tum or cells. Responses to cyto- Many enzym eâ€”prodrug GDEPT system s have been de- toxic chem otherapy indicate that cures of solid tum ors scribed in the past decade Table 1. Two GDEPT system s can only be achieved if a sufficiently high concentration that have been investigated extensively are the herpes of the anticancer drug can be delivered to the m alignant simplex virus thymidine kinaseâ€”ganciclovir HSV-TKâ€”GCV cells. However, achieving this high concentration is often combination, and the cytosine deaminaseâ€”5-fluorocytosine difficult owing to the lack of selectivity of the drugs, CDâ€”5-FC com bination; both have been tested in clinical which also predom inantly accounts for their toxicity to trials. Suicide gene therapy approaches using We have developed a suicide gene therapy based on the deactivated drugs are known as gene-directed enzym e bacterial enzym e carboxypeptidase G2 CPG2 , a bacterial prodrug therapy GDEPT or gene-prodrug activation enzym e isolated from Pseudomonas aeruginosa [9]. Virotherapy involves the use of the advantage over the well-studied suicide genes HSV-TK David Kirn and replication-selective oncolytic viruses [8]. In the acid CMDA , which releases a cytotoxic alkylating agent Hammersmith Hospital, first step, a gene encoding a foreign enzym e is delivered following activation by CPG2. DuCane Road, London, to the tum or for expression. After gene delivery, prodrug adm inis- failed to be sensitized by intracellular expression of CPG2 Centre for Cancer tration must be delayed to enable tim e for expression of as it was discovered that the prodrug could not gain ac- Therapeutics, Institute of the gene in the targeted cells. The catalytic activity of the cess to the cytosol. To overcom e this problem , CPG2 was Cancer Research, expressed enzym e must be sufficient for activation of the expressed such that it was tethered to the outer surface of 15 Cotswold Road, Sutton, prodrug. S 02 http: Table adapted from [4,6,7]. Thus, surface-tethered enzymes appear to have stCPG2 Q 3, the higher the concentration of enzym e advantages in com parison with intracellularly expressed activity in the tum ors, even after long tim e periods [12]. There was also a good correlation between the concen- The CMDA prodrug exhibits lim ited system ic toxicity tration of enzym e in the tum or and the decrease in tum or as dem onstrated by m inim al body weight loss. This volum e after CMDA treatm ent. There is also a substantial bystander effect in leak out of the tum or once form ed. In the untreated controls, it was shown that m ixtures of stCPG2 Q 3-expressing and non-expressing http: To obtain evi- tum or selectivity have also been characterized and include dence of the bystander effect, the proportion of apoptotic reovirus, autonom ous parvoviruses, Newcastle disease cells in the tum ors was m easured. Here, we review the discovery and developm ent established. Tum ors were excised after treatm ent with of replication-selective oncolytic adenoviruses. Genetic three courses of the prodrug CMDA. These data indicate the existence of a substantial bystander effect Approaches to optimizing tumor-selective because the proportion of cells expressing stCPG2 Q 3 adenovirus replication was much lower than the percentage of apoptotic cells. In Two main approaches are currently being used to engineer addition, adm inistration of prodrug resulted in cures in tumor-selective adenovirus replication. The first is to limit all

the groups in which the stCPG2 Q 3 enzym e was the expression of E1A or other early gene products to present. E1A functions to stimulate S-phase stCPG2 Q 3, where four out of six tum ors were cured entry and to transactivate both viral and cellular genes that and the m ean growth delay was 90 days. Substantial are crucial for a productive viral infection [14]. A second reductions in tum or size were observed even when only broad approach to optimizing tumor selectivity is to delete a sm all proportion of cells adm inistered expressed gene functions that are crucial for efficient viral replication stCPG2 Q 3. Moreover, cures were effected in each of the in normal cells but are expendable in tumor cells. A sim ilar approach has been pursued One such interesting class of vectors are the replication- by other groups using tissue-specific prom oters e. The latter approach is discussed in the following section. A second general approach is to com plem ent loss-of- function mutations in cancers with loss-of-function mu- Virotherapy tations within the adenovirus genom e. Many of the sam e Viruses have evolved to infect cells, replicate, induce cell crucial regulatory proteins that are inactivated by viral death, release viral particles and, finally, to spread in gene products during adenovirus replication are also in- hum an tissues. Replication of viruses in tum or tissue activated during carcinogenesis [17â€”20]. Because of this leads to am plification of the input dose at the tum or site, convergence, the deletion of viral genes that inactivate while a lack of replication in norm al tissues can result in these cellular regulatory proteins can be com plem ented efficient clearance and reduced toxicity. Revolutionary by genetic inactivation of these proteins within cancer advances in m olecular biology and genetics have led to a cells [21,22] Fig. These ad- et al. Replication-selective oncolytic viruses virotherapy are Two adenovirus deletion mutation approaches have being developed as a novel, targeted form of anticancer subsequently been described. Over the past decade, several genetically-engineered E1A-CR2 region deletion mutants viruses have been developed, including adenoviruses, her- Mutants in the E1A conserved region 2 CR2 are defective pes viruses and vaccinia Table 2. Viruses with inherent in pRB retinoblastom a protein binding [25,26] as the S70 http: These viruses are being eval- already had inhibited or lost p53 function. Although the precise role of p53 in the wildtype RB protein into a tum or cell line lacking func- replication-selectivity of dl has been variable in vitro tional pRB; both in vitro and in vivo efficacy were dem on- [32â€”34], this virus has proven to be extrem ely selective strated [28]. We designed and im ple- E1BkD gene deletion mutant: The goal of this approach deletion of a gene encoding a pbinding protein, was to increase sequentially system ic exposure to the http: No objective responses were documented with single-agent therapy in phase I or p14ARF Iâ€”II trials in patients with pancreatic, colorectal or ovarian carcinomas. A favorable and potentially synergistic interaction with chemotherapy was discovered in some tumor Mdm2 types and by different routes of administration [37]. A sim ilar phenotype resulted from overexpression of the E Potency can also be im proved by loading viruses with therapeutic genes e. Viral coat m odifications m ight be beneficial if inadequate coxsackie and adenovirus Figure 1. Following dem onstration of safety particular tum or types [45,46]. Im proved system ic deliv- with adenoviral gene and biological activity by the intra-tum oral route, trials ery might require novel formulations or coat modifications, products were sequentially initiated to study intra-cavitary instil- as well as suppression of the humoral immune response. The adenoviral proteins blue lation initially intra-peritoneal , intra-arterial infusion Determination of the viral genes e. E3-region and im- boxes target multiple initially hepatic artery and eventually intravenous ad- mune response parameters mediating efficacy and toxicity components of these pathways at sites upstream of p53, m inistration. Clinical trials of com binations with chem o- will lead to immunomodulatory strategies. Finally, identifi- downstream of p53 and at the therapy were initiated only after the safety of dl as a cation of the mechanisms leading to the potential synergy level of p53 itself. Examples of pregulated cell functions are single agent had been docum ented by the relevant route between replicating adenoviral therapy and chemotherapy shown red boxes. Arrows indicate particles by intra-tumoral, intra-peritoneal, intra-arterial GDEPT represents a rational technology to improve drug se- a positive impact on the and intravenous routes [36]. Toxicity in the liver or other lectivity for cancer cells. Flu-like symptoms fever, rigors, asthenia were the most systems that are under scrutiny or in clinical trials. Several common toxicities and were increased in patients receiv- advantages can be defined: Acute

inflammatory cytokines cancer cells, amplification effects, and bystander cell death. Neutralizing antibodies before reaching therapeutic success. Thus, the main require- increased in all patients, regardless of dose, route or tumor ment for the future is efficient targeting and delivery. Viral replication was documented in head and neck Replicating adenoviruses can achieve higher efficien- and colorectal tumors following intratumoral or intra- cies of delivery com pared with other vectors and can also arterial administration [35]. Neutralizing antibodies did achieve enhanced specificity in certain types of cancer. Increasing that can be augm ented by standard chem otherapeutic agents. Science , â€" Acknowledgements 24 Mineta, T. Cell 56, 67â€"75 ble: Oncogene 19, 2â€"12 29 Scheffner, M. References 88, â€" 1 Zhang, W. Cell 92, â€" have we done and where are we going? Science 3 Denny, W. Science , 4 Niculescu-Duvaz, I. Patents 9, 34 Harada, J. Gene Therapy 8, 89â€"98 10 Marais, R. Cell â€" 33, â€" 12 Stribbling, S. Blood 94, â€" cancer with targeted germ warfare. Virology , â€" thym idine kinase. Oncogene 6, â€" Science , â€" 19 Sherr, C. Science , â€" 46 Douglas, J.

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2: - NLM Catalog Result

Chapter 18 Suicide Gene Therapy Silke Schepelmann, Ion Niculescu-Duvaz, and Caroline J. Springer Introduction A major advance of the 20th century was the deciphering of.

Methods in Molecular Medicine, Vol. Methods and Reviews Edited by: Introduction to the Background and Principles of Suicide Gene Therapy Chemotherapy is widely used with surgery and radiotherapy for the treatment of malignant disease. Selectivity of most drugs for malignant cells remains elusive. Unfortunately, an insufficient therapeutic index, a lack of specificity, and the emer- gence of drug-resistant cell subpopulations often hamper the efficacy of drug thera- pies. Despite the significant progress achieved by chemotherapy in the treatment of disseminated malignancies, the prognosis for solid tumors remains poor. A number of specific difficulties are associated with the treatment of solid tumors, where the access of drugs to cancer cells is often limited by poor, unequal vascularization and areas of necrosis. The histological heterogeneity of the cell population within the tumor is an- other major drawback. Attempts to target therapies to tumors have been addressed by using prodrugs activated in tumors by elevated selective enzymes and are described in Chapter An alternative strategy that use antibodies to target tumors with foreign enzymes that subsequently activate prodrugs is described in Chapter One approach aimed at enhancing the selectivity of cancer chemotherapy for solid tumors relies on the application of gene therapy technologies. Gene therapies are tech- niques for modifying the cellular genome for therapeutic benefit. In cancer gene therapy, both malignant and nonmalignant cells may be suitable targets. The latter approach, known as suicide gene therapy, gene-directed enzyme prodrug therapy GDEPT 1,2 , virus-directed enzyme prodrug therapy 2 Niculescu-Duvaz and Springer VDEPT 3 , or gene prodrug activation therapy GPAT 4 may be used, in isola- tion or combined with other strategies, to make a significant impact on cancer treat- ment. The terms suicide gene therapy and GDEPT can be used interchangeably to de- scribe a two-step treatment designed to treat solid tumors. In the first step, the gene for a foreign enzyme is delivered and targeted in a variety of ways to the tumor where it is to be expressed. In the second step, a prodrug is administered that is activated to the corresponding drug by the foreign enzyme expressed in the tumor. Ideally, the gene for the enzyme should be expressed exclusively in the tumor cells compared to normal tissues and blood. The enzyme must reach a concentration sufficient to activate the prodrug for clinical benefit. The catalytic activity of the expressed protein must be adequate to activate the prodrug under physiological conditions. Because expression of the foreign enzymes will not occur in all cells of a targeted tumor in vivo, a bystander effect BE is required, whereby the prodrug is cleaved to an active drug that kills not only the tumor cells in which it is formed but also neighboring tumor cells that do not express the foreign enzyme 5. The main advantages of optimised suicide gene therapy systems are as follows: Increased selectivity for cancer cells, reducing side effects. Higher concentrations of active drug at the tumor, compared to the concentrations acces- sible by classical chemotherapy. Tumor cell enzyme transduction and kill may induce immune responses that enhance the overall therapeutic response. Prodrugs are not required to exhibit intrinsic specificity for cancer cells; they are designed to be activated by the foreign enzymes, which is technically easier to achieve. A number of hurdles are still to be overcome. The most important are the following: The vectors for gene transduction that target the tumor and achieve efficient infection of cancer cells. Ideally, the vectors should be also nonimmunogenic and nontoxic. The control of gene expression at the tumor. These issues will be addressed in this chapter and in Chapters 2â€”8 on vectors and should be read in conjunction with reviews on the background and principles of GDEPT 6â€”8 , viral vectors 9â€”15 , and nonviral vectors 16,17 , the kinetics of activation 18 , enzymes for GDEPT 19 , the BE 20 , and prodrugs designed for GDEPT 21â€” Herein we summarize the state of the art of suicide gene therapy highlighting recent progress and the areas that to date have hampered the development of suicide gene therapy. They should have high catalytic activity preferably without the need for cofactors , should be different Introduction to Suicide Gene Therapy 3 from any circulating endogenous enzymes, and

should be expressed in sufficient con- centration for therapeutic efficacy. The enzymes proposed for suicide gene therapy can be characterized into two major classes. The first class comprise enzymes of nonmammalian origin with or without human counterparts. Those enzymes that do have human homologs have different structural requirements with respect to their substrates in comparison to the human counterparts. Their main drawback is that they are likely to be immunogenic. The second class of enzymes for suicide gene therapy comprises enzymes of human origin that are absent from or are expressed only at low concentrations in tumor cells. The advantages of such systems resides in the reduction of the potential for inducing an immune response. However, their pres- ence in normal tissues is likely to preclude specific activation of the prodrugs only in tumors unless the transfected enzymes are modified for different substrate requirements. The genes can be engineered to express their product either intracellularly or extra- cellularly in the recipient cells 1. The extracellularly expressed variants are either tethered to the outer cell membrane 1,24 see also Chapter 14 or secreted from cells see Chapter There are potential advantages to each approach. Where the enzyme is intracellularly expressed, the prodrug must enter the cells for activation and, subse- quently, the active drug must diffuse through the interstitium across the cell mem- brane to elicit a BE. Cells in which the enzyme is expressed tethered to the outer surface or secreted are able to activate the prodrug extracellularly. A more substantial BE should therefore be generated in the latter system, but spread of the active drug into the general circulation is a possible disadvantage 1, The basic prodrug and drug requirements of a suicide gene therapy system are briefly described herein. Good pharmacological properties, good pharmacokinetic properties of prodrugs, low cytoxicity of prodrugs with high cytotoxicity of the activated drugs, and effective activation of prodrugs by the expressed enzyme are all important features. Prodrugs should be chemically stable under physiological conditions and be highly diffusible in the tumor interstitium. The released drugs should be as potent as possible, highly dif- fusible, ideally active in both proliferative and quiescent cells, and induce BEs. The activation of the prodrugs is a key step in suicide gene therapy. It is an advan- tage if the expressed enzyme can activate the prodrug directly to the drug, without the need for additional steps requiring further catalysis, because it is possible for the host endogenous enzymes needed for the latter steps to become defective or deficient in cancer cells. The directly linked prodrugs can be defined as a pharmaco- logical inactive derivative of a drug, which requires chemical transformation to release the active drug. In terms of anticancer activity, the conversion of the prodrug to an active drug results in a sharp increase in its cytotoxicity. In a directly linked prodrug, the active drug is released directly following the activation process see Chapter 9. A self-immolative prodrug can be defined as a compound generating an unstable intermediate which, following the activation process, will extrude the active drug in a number of subsequent steps. The most important feature is that the site of activation is normally separated from the site of extrusion. The activation process remains an enzy- matic one. However, the extrusion of the active drug relies on a supplementary spon- taneous fragmentation. Potential advantages of self-immolative prodrugs are the possibility of altering the lipophilicity of the prodrugs with minimal effect on the acti- vation kinetics and the possibility to improve unfavorable kinetics of activation as a result of unsuitable electronic or steric features of the active drug. The range of drugs that can be converted to prodrugs is greatly extended and is unrestricted only by the structural substrate requirements for a given enzyme. A large number of enzymeâ€”prodrug systems have been developed for GDEPT in the recent years and are summarized in Table 1. Quantitative Data In order to compare different GDEPT systems in terms of therapeutic efficiency, each system should be characterized by relevant quantitative parameters. Some parameters refer to the activation process that can be described by kinetic parameters K M , V max , and k cat see Table 2. The concentration of the drug and the rate at which it is released at the activation site depends on the kinetic parameters of the enzymeâ€” prodrug system. Often, published V max and K M values are not compared under equiva- lent conditions, whilst measuring the maximum velocity of the activation reaction and the concentration of substrate needed to reach half of this maximum velocity. As a rule, however, a low K M and high V max or k cat would be expected to favor the systems. The comparison of the yeast CD with bacterial CD bears out this prediction. The yeast

enzyme, which proved to be more effective than its bacterial counterpart in GDEPT experiments, exhibits lower K M and higher V max than the bacterial homolog see Table 2. Unfortunately, comparable values for the V max of these enzymes cannot be obtained because the V max has been determined un- der very different experimental conditions for the various systems and is expressed in different ways, making direct comparisons impossible. The turnover number, k cat , provides additional information about the reaction rate, but the implications of this measure for tumor cell killing is unclear, because it is not yet known if sudden release of the active drug is more effective than Introduction to Suicide Gene Therapy 5 a slow, constant release or if quiescent and proliferating cells differ in their sensitiv- ity to drugs released at different rates. These are the potential of activation of a given system and its degree of activation. The first parameter is defined as the ratio of the IC 50 of the prodrug to the IC 50 of the re- leased drug in a control nontransfected cell system. It represents the maximum possible efficiency of a given enzymeâ€"prodrug system towards a cell line. The degree of activa- tion is defined as the ratio of the IC 50 of the prodrug in the nontransfected cell line to the IC 50 of the prodrug in the transfected or infected cell line and demonstrates the efficiency of the system in a cell line These parameters allow a fair comparison between suicide gene therapy systems in vitro and should also be helpful in designing new systems. However, a number of new systems have been reported in the last three years and will be briefly reviewed here. At physi- ological pH, IAA is activated by HRP to a long-lived species radical that is able to cross cell membranes, and has significantly increased cytotoxicity than the prodrug. This system is claimed to be active against T24 bladder carcinoma cells in vitro A potent BE was reported. However, its use was hampered by the low expression of tyrosinase transgenes in nonmelanotic cells and by the low activity of the enzyme. Recently, mutants of tyrosinase, which accumulate in various cellular compartments the wild- type enzyme is present only in melanosomes , overcome these difficulties. Expression of the mutated enzyme was induced by transfection of human tumor cells 9L gliosarcoma, MCF-7 breast adenocarcinoma, and HT fibrosarcoma. Reduced forms â€" â€" â€" DT-D rat E09, etc. Springer Vectors in Suicide Gene Therapy 29 2 Introduction to Vectors for Suicide Gene Therapy Caroline J Springer 1 Introduction Suicide gene therapy requires vectors or vehicles capable of efficient and selective gene delivery of the therapeutic genes to tumor cells A number of vector systems has been proposed for gene therapy These include: Reviews Edited by Caroline J.

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3: suicide gene therapy, methods and reviews

Suicide gene therapy, also termed gene-directed enzyme prodrug therapy (GDEPT), is a way to improve cancer chemotherapy by selectively activating prodrug in tumors. The gene expressing the enyme is transduced into the cancer cell using a vector orz.

Kirn, Editor Caroline J. Cancer chemotherapy encompasses a large number of The genes can be engineered to express their prod- well-documented and clinically established methods ucts either intracellularly or extracellularly in the for the treatment of malignant diseases. However, the recipient cells 6. There are potential advantages to efficacy of these approaches is often hampered by an each approach. When the enzyme is intracellularly insufficient therapeutic index, lack of specificity, and expressed, the prodrug must enter the cells for acti- the emergence of drug-resistant cell subpopulations. Cells in which the enzyme is application of gene therapy technologies. A more sub- lular genome for therapeutic benefit. In cancer gene stantial BE could therefore be generated with extra- therapy, both malignant and nonmalignant cells may be suitable targets. This approach has two alternatives: The latter approach, known as gene-directed enzyme prodrug therapy GDEPT 1, 2 or virus-direct- ed enzyme prodrug therapy VDEPT 3 , may be used in isolation or combined with other strategies, such as the biotherapies described elsewhere in this Perspective series. VDEPT using selectively replicating viruses as vectors represents a promising means to target suicide genes specifically to tumor cells, an approach that is only beginning to be explored for examples, see Her- miston, this Perspective series, ref. In the first step, the gene for a foreign enzyme is delivered and targeted in a vari- ety of ways to the tumor where it is to be expressed. In the second step, a prodrug is administered that is selectively activated to the drug by the foreign enzyme expressed in the tumor. Ideally, the gene for the enzyme should be expressed exclusively in the tumor cells and should reach a concentration suffi- cient to activate the prodrug for clinical benefit. The catalytic activity of the expressed protein must be adequate to activate the prodrug under physiologi- cal conditions. Because expression of the foreign Figure 1 enzymes will not occur in all cells of a targeted GDEPT, a form of suicide gene therapy, aims to maximize the effect of a tumor in vivo, a bystander effect BE is required, toxic drug and minimize its systemic effects by generating the drug in situ whereby the prodrug is cleaved to an active drug that within the tumor. In the first step in this procedure, the gene for an exoge- kills not only the tumor cells in which it is formed, nous enzyme is delivered and expressed in the tumor cells. Subsequently, but also neighboring tumor cells that do not express a prodrug is administered that is converted to the active drug by the for- the foreign enzyme 5. Beneficial immune effects may be Parameters that influence the success of GDEPT systems induced either by stimulation of the host immune sys- Effective tumor destruction with GDEPT depends on tem or by the use of additional cytokine gene therapy the design of the gene-therapy vectors, the chemistry of see article on immunomodulation by Agha-Moham- the prodrugs and their toxic metabolites, and the madi and Lotze, this Perspective series, ref. The effi- means to deliver one or both components specifically ciency of the BE is another key determinant of the suc- to target cells. Vectors, the vehicles in which the trans- cess of these systems. The specificity of targeting gories. The first comprises foreign enzymes of non- to cancer cells and efficient transfection are essential mammalian origin, with or without human homo- for effective GDEPT, as are the toxicity of the vector logues. Examples are viral tyrosine kinase TK , bacterial and the uptake of prodrugs or drugs by normal and cytosine deaminase CD , carboxypeptidase G2 CPG2 , malignant cells. With the exception of logues of enzymes in the first category have different cyclophosphamide, ifosfamide, and some prodrugs substrate structural requirements than the foreign designed for CPG2 and NR, none of the prodrugs enzymes have. Their main disadvantage as therapeutic shown are self immolative. Self-immolative prodrugs agents may be the potential to elicit an immune response derived from alkylating agents and anthracyclines in humans, although this may actually provide benefits have been synthesized for activation by CPG2 12, to therapy. Enzymes of the second category are unlikely 13 , and self-immolative derivatives from secocyclo- to induce immune responses, but

their use will in most propylindolines and ene-diyne prodrugs have been cases lead to some prodrug activation in normal cells. The second step is administration of the prodrug. The first parameter is defined as the ratio situ in the target tumor. In drug system toward that cell line. The degree of activa- addition, they should be chemically stable under phys- iological conditions and diffuse readily in the tumor interstitium. They should also have good pharmaco- logical and pharmacokinetic properties, and release an active drug with a good BE. Prodrugs must also be tai- lored to their site of activation: The cytotoxicity differential between the prodrug and its corresponding drug should be as high as possible, and the active drug should be highly diffusible or be actively taken up or exported by cells. The design of a prodrug that can release a highly effective drug requires knowledge of the quantitative structure-activity rela- tionship QSAR. For this reason, and in order to obtain proof of principle for GDEPT, most of the prodrugs used in suicide gene therapy to date have been clinical- ly licensed anticancer agents with known pharmaco- logical, pharmacokinetic, dosage, and safety parameters. A self-immolative prodrug can be defined as a com- pound that generates an unstable intermediate that then extrudes the active drug in subsequent steps. Although the activation process that generates the unstable species is generally enzyme-mediated, extru- sion occurs spontaneously through the fragmenta- tion of the prodrug, often at a distinct site. Self- immolative prodrugs allow their lipophilicity to be altered with minimal effect on the activation kinetics. Indeed, kinetics of activation that are unfavorable due to the chemical or steric features of the active drug can be improved by this approach. The range of drugs that can be converted to prodrugs is greatly extended, Figure 2 Self immolation of prodrug1 to yield the chemotherapeutic drug dox- and is unrestricted by the structural substrate require- orubicin. The doxorubicin prodrug 1 is cleaved by carboxypeptidase G2 ments for a given enzyme. Figure 2 shows one self- CPG2 , releasing the glutamic acid 3 and an unstable intermediate 2. This scheme can be 12, 13 , followed by the spontaneous extrusion of the readily modified to allow the production of structurally similar drugs, DNA-damaging agent doxorubicin. The Journal of Clinical Investigation May Volume Number 9 tion is defined as the ratio of the IC50 of the prodrug in dase A to improve the efficiency of this enzyme toward the nontransfected cell line to the IC50 of the prodrug specific substrates that, by design, are less prone to in the transfected or infected derivative of the cell line interfere with other human peptidases This approach requires the cotransfection of immediately and quantitatively to the active form of genes for each of the enzymes, but is expected to increase the drug, the degree of activation is identical to the the overall yield of the desired final metabolite, the active potential of activation for that GDEPT system. In the case of GCV, Blanche et al. As a rule, however, low Km and high Vmax or kcat would As discussed above, the potential of activation of a be expected to be found in relatively effective systems, GDEPT system reflects its maximal theoretical effi- and the comparison of the yeast CD with bacterial CD ciency, at least toward a specific cell type. Unfortunate- bears out this prediction. As shown in Table 1, the ly, not all the systems can be defined in this way, yeast enzyme, which proved to be more effective than because multiple products may be released, and the its bacterial counterpart in a GDEPT experiment, toxicity of each of these metabolites may not be avail- exhibits lower Km and higher Vmax values Thus, although GCV is relatively nontoxic, its er, the literature does not supply consistent values for monophosphorylated derivative, GVCMP, is highly the Vmax of these enzymes, because Vmax has been cytotoxic 19 , so the potential of activation of this sys- determined under very different experimental condi- tem cannot be calculated accurately from the known tions for the various systems and is expressed in dif- IC50 of the triphosphorylated derivative, GCVTP. Like- ferent ways, making direct comparisons impossible. The the final active metabolite. Some highly cyto- escent and proliferating cells differ in their sensitivi- toxic compounds with IC50 in the nM range such as ty to drugs released at different rates. Some of these approaches build cytotoxicity prodrugs. An alternative is to ity is especially useful for tailoring the prodrug for use modify the active site of the enzyme by site-directed with an extracellular or intracellular activating enzyme. By definition, it must be TK with improved kinetic parameters for the prodrugs lower than or at least equal to the potential of activation GCV and ACV. Similarly, Smith and colleagues 16 for the system, as is seen for all the systems analyzed in performed site-directed mutagenesis on carboxypepti- Table 1. Their

values offer some insight into the in treated become tumor-free, but when tumor-bearing vitro situation, where a single cell type, transfected with mice were administered lovastatin or apigenin during a gene for an activating enzyme, is challenged with the GCV treatment, their antitumor response rate doubled. For several reasons, these values may not esis that gap junctions mediate the BE after GCV treat- accurately reflect the situation in vivo. Additional fac- ment, other data suggest that additional mechanisms tors, such as pharmacokinetics, prodrug distribution, are involved. In one study of human lung tumor cell and immune responses complicate the overall picture. In this sys- cell types, and not all cells in a tumor may be accurately tem, gap junction communication was not apparent modeled by the cell line chosen for in vitro study. However, Increasing the BE neither an inhibitor 1-octanol nor an enhancer all- The extent of the BE can be determined from the effect trans retinoic acid of gap junction communication of the treatment on nonâ€”genetically modified cells that affected the extent of the BE, suggesting either that low takes place after prodrug administration, when only a levels of gap junctions can produce a maximal BE or fraction of the tumor mass is genetically modified to that bystander cell killing occurs by other means The striking suc- Boucher et al. This mechanism is postulated for Another suggestion is that the TK enzyme is trans- 5-FU formed from 5-FC; for the metabolites of CP and ported by apoptotic vesicles or through gap junctions. IP, aldophosphamide, phosphoramidic mustards, and Phagocytosis of material e. Supporting this model for the BE is tosis was detected in bystander cells and it was found the observation that cell-to-cell contact is not required that this event could be inhibited by BCL2 expression. It has also been ulation by 5-FC. This BE is dramatically greater in vivo, suggested that killing of tumor cells by apoptosis could even with immunocompromised mice: Consistent with this model, one report caused by the BE, expressed as a percentage of the max- showed that tumor cells resistant to BE did not show imum measured decrease. Furthermore, dieldrin, a drug known to single data point for the BE. The TE50 in the cell lines decrease gap junction communications, diminished the ranged from 0. Although by pharmacological manipulation of the gap junctions the BE has been observed in immunocompromised in vivo. Apigenin, a flavonoid, and lovastatin, an animals, recent findings suggest that the BE in vivo is inhibitor of HMG-CoA reductase, both upregulate gap mediated largely through the release of cytokines. In a similar experiment, when HSV-TK was The Journal of Clinical Investigation May Volume Number 9 transfected into cells grown as xenografts, the tumor the enzymes, to maximize drug release or the BE, to growth was inhibited for up to 50 days in GCV-treat- take advantage of self-immolative strategies of acti- ed, immunocompromised nude mice, but failed to vation, or to allow the active drug to accumulate more eliminate all the tumor cells in these animals, and readily in tumor cells. Finally, it will also be useful to tumors regrew 40â€”50 days after implantation. Taken therapy or immunotherapy has previously been sug- together, these studies strongly suggest that an intact gested. The transfection of suicide IL-2 appears to be critical for immune-mediated genes together with genes that are able to increase the tumor suppression in this system. In one experiment, sensitivity of the tumors to radiation or enhance the cells grown as xenografts in syngeneic mice were potential of the host immune system is an alternative injected with an adenoviral vector containing the HSV strategy. Whereas the tumors continued to cating oncolytic adenoviruses, such as ONYX grow in the animals injected with a control vector or 32; see also Heise and Kirn, this Perspective series, ref. However, only animals tive series, ref. The combination of these replicat- treated with both genes developed effective systemic ing adenoviruses with conventional chemotherapy antitumoral immunity against tumorigenic rechal- has proven highly effective, and replacing the lenges. These and similar findings 30 Institute of Cancer Research. We would like to thank P. Expression of the bacterial nitroreductase enzyme in mammalian cells renders them selectively sensitive to killing become a clinically efficient treatment of cancers. Major by the prodrug CB Advanced Drug Delivery Reviews. Gene delivery from replication-selective viruses: Double suicide gene therapy, Metabolism of 5-fluorocytidine to 5-fluorouracil in human colorectal in which a combination of suicide genes is introduced tumor cells transduced with the cytosine deaminase gene: The antitumor effects when only a small percentage of tumor cells express released active drugs in such an approach can act by dif- cytosine deaminase. A cell

surface tethered enzyme improves efficien- ferent mechanisms, leading to a synergistic effect on cy in gene-directed enzyme prodrug therapy. The design of selectively-activated anti-cancer prodrugs for use in antibody-directed and gene-directed released together. Additionally, the occurrence of resist- enzyme prodrug therapies. Improving enzymes for human carcinoma cell lines grown in vivo that double gene therapy. Patent property of prodrug allowed the elimination of tumors, but neither gene involving gene therapy Expert Opinion on Therapeutical Patents. Future developments in this technology

4: JCI - Prodrug-activating systems in suicide gene therapy

Gene therapy has expanded rapidly over the last decade. The number of clinical trials reported by included protocols and patients. Phase I trials predominate with trials of patients versus Phase II (57 trials with patients) and Phase III (3 trials of patients).

5: Ion Niculescu-duvaz - www.enganchecubano.com

Prodrug-activating systems in Perspective suicide gene therapy SERIES On cancer biotherapy David H. Kirn, Editor Caroline J. Springer and Ion Niculescu-Duvaz Cancer Research Campaign Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, United Kingdom Address correspondence to: Caroline J. Springer, Cancer Research Campaign Centre for Cancer Therapeutics at the.

6: JCI - Citations to Prodrug-activating systems in suicide gene therapy

Caroline J. Springer and Ion Niculescu-Duvaz Cancer Research Campaign Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, United Kingdom Address correspondence to: Caroline J. Springer, Cancer Research Campaign Centre for Cancer Therapeutics at the Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey.

7: The emerging fields of suicide gene therapy and virotherapy | Ion Niculescu-duvaz - www.enganchecuba

Ion Niculescu-Duvaz The foreign enzym e needs to be expressed exclusively or Expression of CPG2 in the cytoplasm (CPG2) of a variety and Caroline J. with a relatively high ratio in tum or cells com pared with of tum or cell types sensitizes the cells to CMDA [10].*

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