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Tumour-targeted drug and gene delivery: principles and concepts

Jim Cassidy and Andreas G. Schätzlein

Delivery systems for tumour targeting fall into two basic categories: drug conjugate systems, in which individual drug molecules are chemically modified to target them directly to the tumour; and carrier-based systems, in which the drug or gene is first packaged non-covalently into a synthetic carrier that is then targeted to the tumour. In both cases, the objective is to maximise exposure of the target cells to the drug yet minimise side effects that result from nonspecific toxicity in normal tissues. The creation of such dose differentials is based on phenotypic differences between the tumour and the rest of the body. However, although a wide range of such changes have been linked to the transformation of normal cells to cancer cells, no single common feature exists to allow unambiguous targeting to the tumour. In addition, the tumour microenvironment creates physical barriers that significantly impair transport within the tumour. It is therefore important to match the delivery requirements of the drug to the capabilities of the delivery system. In this review, a brief overview is given of the underlying concepts and principles that help guide the development of such tumour-targeting strategies.

The idea of a drug as the magic bullet, originally suggested at the end of the 19th century by Nobel Laureate Paul Ehrlich, has provided the paradigm for drug targeting. Pharmacologists have striven to develop drugs that avoid the sometimes dramatic and even life-threatening side effects of anticancer therapy that are often synonymous with 'chemotherapy' in the public's mind. A good example of this is alopecia induced by chemotherapy, which is an obvious side effect with significant associated psychosocial morbidity. Directing the drug away from the hair

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follicle would thus represent a significant therapeutic improvement.

Over the years, improved administration modalities and novel cytotoxic drugs have led to significant improvements in the management of cancer (Refs 1, 2, 3). However, it is the increasing understanding of the molecular and genetic basis of cancer that promises to change fundamentally the way the disease will be treated (Ref. 4). In addition, the advent of drugs with clearly defined molecular targets and mechanisms of action [e.g. imatinib mesylate/Gleevec™, which targets the Bcr-Abl tyrosine kinase in chronic myeloid leukaemia (Ref. 5)] holds the promise of therapeutic intervention with a high level of specificity that would avoid both the multitude of side effects and the need for toxicity-limited dosing. The improved understanding of the molecular and genetic basis of cancers has also led to the development of promising gene therapy strategies such as replacement of tumour suppressor genes, suppression of oncogenes, enzyme-prodrug therapy (delivery to the tumour of an enzyme capable of converting a non-toxic prodrug into an active drug) and immunotherapy (Refs 6, 7).

New treatment modalities have brought with them new challenges, not least in terms of delivery. The efficient delivery of genetic materials to the target site has in fact been identified as a bottleneck, hampering the progress of gene therapy into the clinic (Ref. 8). The concepts and principles underlying the development of delivery-system-based tumour-targeting strategies, rather than their stage of clinical development, form the basis of this review. Most of the strategies discussed in this review have been extensively explored in preclinical animal models but the stage of clinical development that has been reached varies widely (further details can be found in some of the specialist reviews referenced below).

Considerations, objectives and challenges of tumour targeting

The drug

'All things are poison and nothing is without poison. It is the dose that makes a thing poisonous.' The incisive words of the 16th Century pharmacologist Paracelsus emphasise the fact that, even with the most-specific drugs, there remains the need to control the dose. To achieve therapeutic success, it is crucial to achieve the appropriate exposure of cancer cells to the drug or active agent. Aside from scheduling issues [e.g. taking into account the cell cycle or circadian rhythm (chronotherapy) when administering drugs], the level of anticancer response in general is likely to depend on the concentration achieved at the target over time.

Ideally, one would control the spatiotemporal profile of drug distribution on a cellular level so that all the target cells are exposed to the required concentration of drug for a sufficient length of time, while sparing the rest of the body, thus adding to the specificity of the drug itself. Drug delivery and targeting strategies modulate the distribution profile of a drug in order to create such exposure differentials between target cells and healthy tissues. Such strategies could thus offer an approach to increase drug safety that complements the pharmacodynamic specificity of the drug.

The objectives of targeted delivery to solid tumours are twofold: (1) to increase the total exposure of tumour tissue to the drug or active principle, while (2) limiting the exposure of healthy tissue. The importance of each of these parts will depend on the properties of the active compound: highly specific drugs (e.g. genes controlled by tumour-specific promoters) would be expected to benefit mostly from increased expression in the tumour, whereas efficient but less-specific agents (e.g. toxins) would need to be limited with respect to normal tissue exposure. Therefore, a combination of drug and delivery system needs to be seen as a unit in which the specificity and potency of the active compound is augmented by the capabilities of the delivery system.

Conventional small molecule drugs exert their effects directly after binding to a specific receptor or site, possibly after initial metabolic activation from an inactive prodrug, such as 5-fluorouracil (Ref. 9). The mechanism of action of some novel therapeutic agents tends to be more complex, as these agents often only act indirectly: for instance, gene-based drugs require transcription and translation of the transgene to produce the active protein, whereas other (oligo) nucleic-acid-based drugs such as antisense or short interfering (si)RNA act by interfering with these processes (Refs 10, 11). Such indirect strategies not only give scope to modulate therapeutic specificity by exploitation of the various regulatory elements but also often form part of powerful amplification

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cascades. For example, using cellular gene expression machinery, the production of the therapeutic protein can reach a significant proportion of the total cellular protein starting with only a few nucleic acid molecules, thus potentially making genes one of the most potent of drugs (Refs 12, 13). Although some of the principles that apply to tumour targeting of drugs and synthetic gene delivery systems are equally relevant for viral gene delivery vectors, these will not be considered specifically (for reviews see Refs 14, 15).

Amplification cascades are not only important at the molecular level but analogous concepts also come to bear at the cellular and even systemic level (Ref. 7). Such cascading effects can act on sites upstream of the tumour cells, as is the case with therapies that target the tumour microenvironment such as anti-angiogenic therapies, where destruction of an endothelial cell can cause the subsequent starvation of multiple tumour cells (Ref. 16). The bystander effect represents another important amplification mechanism whereby an active drug produced in one cell also acts on cells in the immediate vicinity, for instance after transport through cell–cell communications. An understanding of bystander and cascading effects is crucial to be able to match the capabilities of the delivery system with the therapeutic strategy: a drug that acts directly as a cytotoxic would need to reach each and every cancer cell in sufficient quantities, whereas a strategy that maximises cascading and bystander effects would only need to reach a proportion of the target cell population.

The tumour

In considering targeting strategies, it is important to realise the constraints that are introduced through the pathophysiology of the tumour or tumour cells that are being targeted. In addition to transformed cells, the tumour comprises several specialised tissues and cells (e.g. blood vessels or connective tissue) that have not been transformed. The interaction of tumour cells with this microenvironment and the surrounding tissues not only plays a major role in controlling tumour growth, angiogenesis, invasion and metastasis (Ref. 17), but also provides unique therapeutic opportunities. For instance, the cells of the tumour vasculature express specific receptor combinations that are potentially useful for targeting (Ref. 18). As a therapeutic target, these untransformed cells have the added advantage of avoiding the potential development of drug resistance that is frequently observed with transformed cells (Ref. 16). However, the microenvironment not only provides potential targets but also creates challenging physical barriers for drug targeting.

In order for a drug or drug delivery system to reach the tumour cell, it first needs to travel in the vascular compartment to the tumour site, cross the wall of the terminal microvessel, distribute through the interstitial space, bind to the target and/or be taken up by the tumour cell. One of the challenges of targeting drugs to solid tumours lies in the physical barriers in the host and tumour that hamper this process, many of which are closely linked with the pathophysiology of tumour growth and development, and in particular its vasculature (Ref. 19).

Tumour vasculature

The rapidly dividing cells in a tumour have a high metabolic turnover and therefore need a constant supply of nutrients and oxygen. Distribution of nutrients by diffusion is limited to the immediate vicinity of the tumour, which therefore needs to recruit more blood supplies in order to continue to grow. Without successful neovascularisation, tumours can only grow to sizes of a few millimetres (Ref. 16). The newly sprouting blood vessels have an atypical morphology: for instance, shunts and tortuous, dilated or giant capillaries reduce the hydrodynamic functionality (Ref. 20), whereas patchy endothelial lining, mosaic vessels (Ref. 21) and various 'holes' in the endothelium (Ref. 22) increase permeability to macromolecules (Ref. 23), and therefore drug carriers (Fig. 1). This effect has been successfully exploited for drug targeting using the so-called enhanced permeability and retention (EPR) effect (see below).

Growth of the tumour in a confined space with lack of lymphatic drainage, increased filtration of proteins and other colloids, and hydrodynamic changes together promote high interstitial pressure in the tumour centre (Refs 24, 25, 26). Moreover, blood flow is heterogeneous throughout the tumour and is changeable over time, creating a range of perfusion states ranging from necrotic to well vascularised (Refs 19, 27). Taken together, these changes to blood flow and pressure reduce convective transport (i.e. transmission by the movement of the

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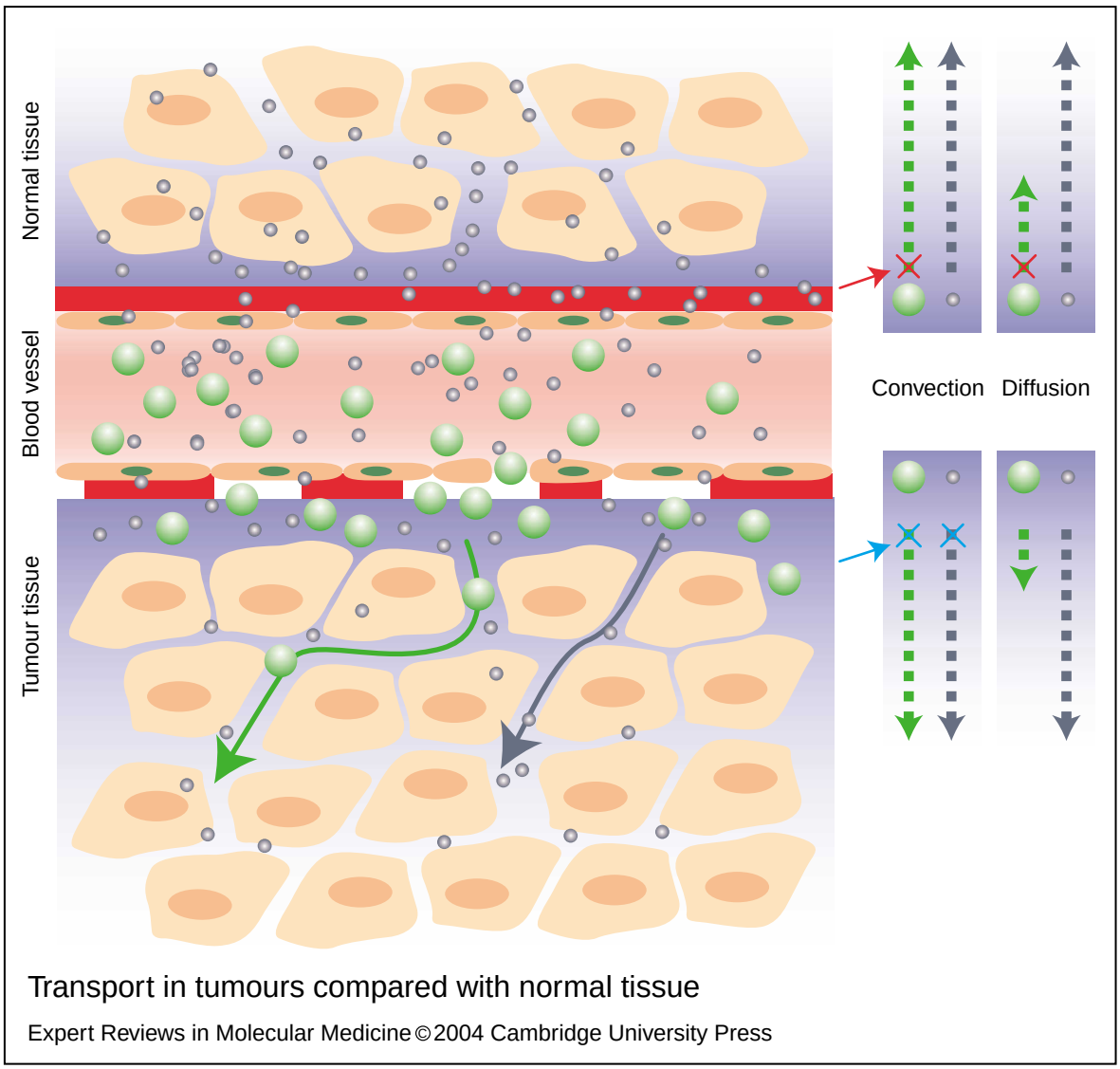

Figure 1. Transport in tumours compared with normal tissue. In normal tissues, the basal lamina and endothelial lining of tumour blood vessels allow extravasation of low-molecular-weight (LMW) molecules (grey) either by convection (transmission of molecules by the movement of the medium) or diffusion (spontaneous movement of molecules by random thermal motion). However, extravasation of high-molecular-weight (HMW) macromolecules and particulates (green) by convection or diffusion is normally prevented in most tissues; this is indicated by the red cross in the right panel. In tumour tissues, the basal lamina and endothelial lining of tumour blood vessels have a leaky morphology, allowing macromolecules and particulates below a 'pore' cut-off size to pass through the vessel walls. After extravasation, these macromolecules and carriers become trapped within the tumour because increased interstitial pressure, dysfunctional hydrodynamics and lack of lymphatic vessels reduce fluid drainage and therefore reduce transport by convection (blue crosses in the right panel) rather than by diffusion. Thus, transport beyond the blood vessels and in the tumour interstitium depends largely on diffusion rather than convection. The efficiency of transport by diffusion depends on the hydrodynamic radius and is therefore better for LMW drugs (as indicated by the longer arrow for LMW molecules than HMW in the right panel). Interstitial transport of large entities is further limited by spatial restrictions owing to the density of the extracellular matrix. As a consequence, the tumour acts like a sieve that filters out suitable carriers from the blood stream: this is the so-called enhanced permeability and retention (EPR) effect. The tumour tissue is illustrated here with extra layers of cells, to represent the longer diffusion distances that might need to be traversed. However, even for LMW drugs, distribution throughout the tumour is not necessarily homogeneous and an equilibrium might only be reached after extended periods of drug exposure (e.g. see Ref. 33).

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medium – in this case blood or interstitial fluid), but not necessarily the initial extravasation, and contribute to the heterogeneity in the distribution of drug molecules and delivery systems throughout the tumour (Fig. 1).

Traversing the interstitial space

Once molecules have extravasated from the vasculature, they need to travel through the interstitial space to reach the tumour cells. The interstitial space between cells is filled with extracellular matrix (ECM), which helps to organise the individual tumour cells into the three-dimensional architecture of the actual tumour tissue. This architecture and the interaction between the ECM and tumour cells has been implicated in tumour growth, differentiation, vascularisation, invasion and metastasis, as well as in drug resistance (Refs 16, 17, 28, 29). As therapeutic carriers or molecules need to be transported from the blood vessel to reach the tumour cell, it has also become clear that the interstitial space plays an important role in determining the clinical success of drug and gene delivery systems (Ref. 24).

The transport of molecules depends both on convection and diffusion. The convective transport of molecules is independent of their molecular weight because the molecules are carried along in the net flow of blood or interstitial fluid. However, one of the effects of the increased interstitial pressure within tumours is a reduced convective flow throughout the tumour, thereby significantly reducing the contribution of convective transport (Fig. 1). By contrast, transport by diffusion does not require fluid flow but depends instead on the magnitude of the concentration gradient and the diffusion coefficient. The diffusion coefficient decreases with increasing hydrodynamic radius. Thus, diffusion-dependent transport decreases with increasing molecular weight of the molecule/carrier (Ref. 30).

Although many traditional drugs have molecular weights of a few hundred Daltons, and thus diffuse readily, biological agents and drug carriers are orders of magnitude larger, making diffusion potentially very slow (Ref. 31). Indeed, the hydrodynamic radius of low-molecular-weight (LMW) dextran (MW 4400) is about 14 times smaller than that of high (H)MW dextran (MW 2000 000) and consequently the diffusion coefficient is around 14 times higher (Ref. 32). In tumours, the composition of the ECM, particularly the high collagen content, further reduces penetration (Ref. 30). An additional frictional component to the reduced diffusion is particularly important for large molecules or carriers for which the hydrodynamic radius approaches the size of the spacing between the matrix elements (Ref. 31). For macromolecules such as IgM and dextran, variations of the diffusion coefficient by a factor of 5–10 have been demonstrated. Although smaller molecules (~50 kDa) were not affected by an increase in tumour collagen content (Ref. 31), diffusion coefficients of particulate carriers such as liposomes have been estimated to vary by up to two orders of magnitude depending on collagen content (Refs 30, 31).

The difficulties of intratumoural transport are illustrated by the fact that even LMW drugs with relatively high diffusion coefficients such as doxorubicin show a heterogeneous distribution even more than 24 hours after intravenous infusion in humans (Ref. 33). Thus, it is not surprising that a pronounced heterogeneity of distribution has also been observed frequently for larger molecules such as antibodies (Ref. 34), macromolecules (Ref. 35) or vesicles (Ref. 36).

Other challenges

Other factors that potentially make targeting to tumours a challenge have been linked to the heterogeneity of the tumour cell population and its ability to change its molecular characteristics (i.e. a mutator phenotype) (Ref. 37). As a consequence, resistance to targeted therapies could develop due to receptor downregulation or loss of expression, or through negative selection that eliminates the most effectively targeted cells first.

Targeted drug delivery systems

The development of targeted delivery systems has benefited from our improved understanding of tumour pathophysiology, particularly as to how tumours differ from healthy tissue, and has led to the development of tools and materials that allow us to exploit these differences. The development of delivery systems is largely based on the engineering of materials on a nanoscale to create carriers that match specific biological and pharmaceutical requirements. Although the range of materials that has been developed for drug delivery and targeting purposes is extremely broad, the range of 'engineering objectives' they attempt to achieve is more limited and relate to

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the type of packaging, the size of the delivery system, its intrinsic and extrinsic properties, and the specificity of targeting.

Packaging

Targeted delivery systems fall into two basic categories: carrier-based systems and drug conjugates (Fig. 2).

The prototypical carrier-based delivery systems consist of non-covalent aggregates of carrier molecules packaging multiple drug molecules: (1) vesicles such as liposomes, which are formed by lipid bilayers that encapsulate water-soluble drug molecules within the aqueous core and/or lipophilic drugs in the bilayer; (2) micelles, which are smaller, amphiphilic aggregates with a solid, lipophilic core; and (3) nanoparticles, which are formed by co-precipitation of drug and carrier (Refs 38, 39, 40). Carrier-based systems package many drug molecules within a single carrier unit, only to be released ideally at the target site or after uptake into the target cell. The drugs are usually non-covalently bound to, or encapsulated within, the carrier, and drug

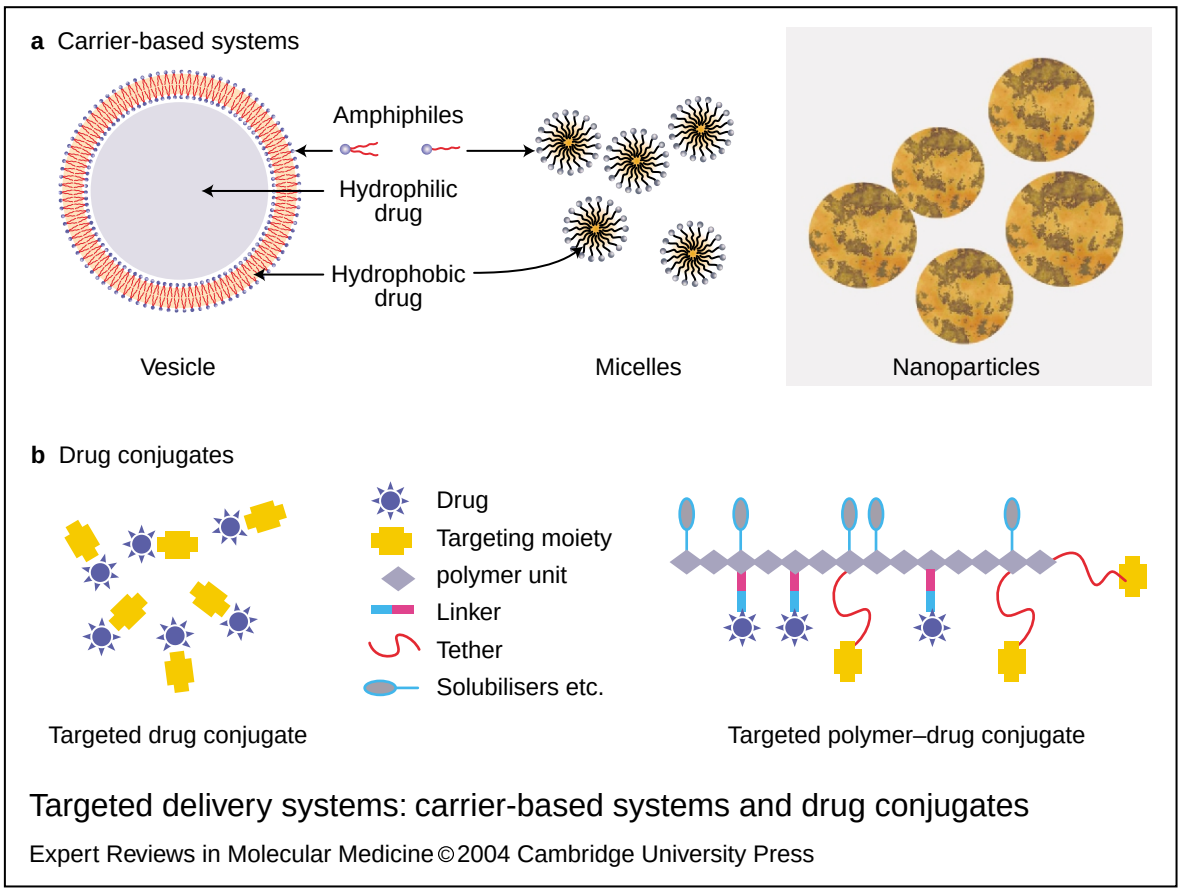

Figure 2. Targeted delivery systems: carrier-based systems and drug conjugates. (a) The carrier-based delivery systems typically consist of non-covalent aggregates of carrier molecules packaging multiple drug molecules. In vesicles, a lipid bilayer encapsulates the soluble, hydrophilic drug molecule within the aqueous core, whereas hydrophobic drugs can be accommodated in the membrane. In micelles, the solid core accommodates lipophilic substances. Vesicles and micelles are both composed of amphiphiles, which are compounds consisting of molecules having a polar, water-soluble group attached to a water-insoluble hydrocarbon chain. Nanoparticles are formed by co-precipitation of drug and carrier (e.g. DNA and cationic polymer). (b) Drug conjugates target drug molecules directly without packing. The targeting ligand is covalently coupled to the drug, making the efficiency of drug loading and uptake comparatively lower than in polymer–drug conjugates. In polymer–drug conjugates, many drug molecules are covalently bound to a single polymer backbone which, in turn, can be targeted to the tumour. Conjugate can be tailored further using cleavable linker, tethers, solubilisers and other functional groups.

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targeting takes place by manipulating the biodistribution of the whole carrier system rather than that of the individual drug molecules. Thus, the biodistribution and potential targeting depends on the properties of the delivery system rather than those of the drug. Moreover, the same delivery system can potentially be used to target different types of drugs to the tumour. An example of this would be the ability of synthetic gene delivery systems to deliver different genes (reporter and/or therapeutic genes, of various sizes) using the same technology (Refs 7, 41).

By contrast, the drug molecules in drug conjugates are not packaged within a carrier but are targeted directly through the covalent coupling to a targeting moiety. An example of this is the combination of antibodies (the targeting moiety) with potent agents such as toxins to create an immunotoxin (Ref. 42). The efficiency of drug loading and uptake per cell-binding event is comparatively lower in these types of simple conjugates as the stoichiometry of ligand to drug is 1:1. Therefore, they are suited for targeting of potent agents to a receptor that is quickly internalised, recycled and expressed in high numbers (preferably) on the target cells. In situations where higher ratios of drug to ligand are required, materials such as polymer–drug conjugates offer an alternative. In these systems, many drug molecules are covalently bound to a single polymer backbone which, in turn, can be targeted to the tumour (Ref. 43). All conjugates have in common that the covalent modification of the drug creates a new chemical entity that might not have the same activity as the original drug. This might have posed a potential disadvantage but, with the development of linkers that are selectively cleavable in certain environments (Ref. 44), this has become an additional tool to increase specificity, for example by utilising bonds that are cleaved by endosomal enzymes and thus limit drug release outside the target cell (Refs 45, 46).

Size matters

An important implication of the packaging of the drug lies in the size of the resulting system. Carriers are usually formed by self-assembly or co-precipitation of huge numbers of small(er) molecules, resulting in particulates with sizes the order of 25 nm to 1 μm (which is equivalent to millions of Daltons) (Ref. 40). Conjugates, even polymer–drug conjugates, are macromolecular by nature and are significantly smaller, with molecular weights from 10^3–10^4 Da (Ref. 43).

Macromolecular drugs and particulate carriers are normally retained within the blood vessels, and only in specialised organs (e.g. spleen, liver) will the endothelium allow extravasation of these materials. However, because of the 'leaky' morphology (see above) of the tumour blood vessels, macromolecules and particulates below a 'pore' cut-off size are able to pass through the vessel walls (Fig. 1). The specific cut-off size depends on the localisation and nature of the tumour and ranges from 0.2 μm to >1 μm, with 400–600 nm being the maximum in the majority of animal models (Refs 3, 47). After extravasation, these macromolecules and carriers become trapped within the tumour because increased interstitial pressure, dysfunctional hydrodynamics and lack of lymphatic vessels reduce fluid drainage and transport by convection (rather than diffusion). As a consequence, the tumour acts like a sieve that filters out suitable carriers from the blood stream. The efficiency of targeting depends on the use of carriers with a long plasma half-life that circulate in the bloodstream for a long time and thus have a higher likelihood of passing though the tumour. This EPR effect has successfully been used for the targeting of drugs to solid tumours and has a broad applicability in various tumour types (Refs 43, 48, 49).

The lack of transport by convection and the tendency for carriers to accumulate around the blood vessels after extravasation in the tumour means that transport beyond the blood vessels and in the tumour interstitium depends largely on diffusion rather than convection. Diffusion is inversely linked to the hydrodynamic radius (Ref. 32); thus, the larger the hydrodynamic radius, the slower the interstitial transport of larger carriers and HMW conjugates, and the more hampered their distribution throughout the tumour. In this situation, it might be advantageous to use small targeted molecules [e.g. nanoconjugates (Ref. 50)], which may have better tissue penetration in diffusion-limited transport situations. Such systems also have disadvantages that could outweigh any advantage but it is currently not clear what combinations of properties would be ideal. It has been postulated for liposomes such as those currently in clinical use that the encapsulated LMW drug is released from perivascular vesicle depots over time to distribute further by diffusion (Ref. 36).

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The molecular weight and size of a delivery system also influences its elimination from the body and thus biocompatibility: molecules larger than ~40–60 kDa (or ~5 nm in diameter), and all particulate carriers, will not be filtrated in the glomeruli of the kidney and will accumulate in the body unless there is hepatic elimination, or cleavage into smaller molecules (e.g. polymers), or disintegration of aggregates (e.g. liposomes). Rapid elimination is one of the potential disadvantages of LMW conjugates, and molecular weight increase by polymer conjugation is in fact a tool that is used to prolong the systemic circulation of certain proteins (Ref. 51).

Intrinsic properties and passive targeting

An ideal targeting system would be a system that was completely inert in the circulation until it reaches the target site. Such a system would lack any nonspecific interactions with cells and endogenous macromolecules and be evenly distributed throughout the body unless made to target a specific site. In reality, neither drugs nor delivery systems are 'inert' in the biological sense. On administration, a multitude of interactions occur with elements of the vascular compartment such as the blood and endothelial cells, as well as with proteins and macromolecules that strongly modify the biodistribution and reduce complex stability (e.g. albumin, IgM, complement, platelets; Refs 52, 53, 54). These interactions are determined predominantly by the physicochemistry of the system.

Some of the physicochemical characteristics of the drug delivery system cannot be changed readily and are said to be 'intrinsic' properties of the carriers (Ref. 55). As described above, size is an example of an intrinsic property that greatly influences biological behaviour but can only be modulated to a limited extent (e.g. particulate systems always remain particulate). Another intrinsic property that is particularly important with respect to synthetic, nonviral gene delivery systems is their particle surface charge. Synthetic gene delivery systems tend to carry a strong net positive charge that makes individual particles repel one another and thus makes the formulation colloidally stable (Ref. 56). This can also be used for targeting purposes: cationic liposomes and liposome–DNA complexes appear to be taken up preferentially by angiogenic endothelial cells, whereas the anionic, neutral or sterically stabilised neutral liposomes are not (Ref. 57). However, a positive charge induces extensive nonspecific binding of negatively charged molecules and particles, which leads to unforeseen changes in their biodistribution and instability of the carriers in the body (Refs 52, 53, 54) and is probably one of the main causes for the difficulties of correlating experimental results in vitro and in vivo. Furthermore, it is likely to override more-specific targeting based on receptor–ligand interactions (Ref. 58). Such targeting of the tumour by virtue of intrinsic carrier properties such as particle size (EPR effect; Refs 43, 48, 49) or charge (angiogenic blood vessels; Ref. 57) is also called 'passive targeting'.

Because it can be difficult to tailor the physicochemistry of delivery systems, it is essential that the biological effects of intrinsic properties be taken into account when devising a targeting strategy. If the intrinsic properties are unfavourable, it might be possible to modulate some of the undesired effects by 'masking' (i.e. hiding them). As the undesired nonspecific interactions occur predominantly via the surface of carrier systems, masking strategies have tended to modify or hide the carrier surface, for instance by introducing a steric barrier of hydrophilic polymers extending from the surface (Refs 51, 59, 60). This creates a zone in which the approach of other macromolecules and cells to the carrier surface, and therefore nonspecific binding or tagging by components of the complement system, is impeded (Ref. 61). This approach has for example been used in 'stealth' liposomes that are currently in clinical use (Ref. 62). The same strategy has also been adopted with macromolecules and therapeutic proteins (Refs 51, 63).

Extrinsic properties and active targeting

A system in which nonspecific interactions with cells and biological molecules have been minimised as far as possible offers a good platform to introduce specific targeting by the addition of extrinsic elements that allow specific interaction with the target site. The use of a ligand for targeting is synonymous with 'active targeting' (as opposed to 'passive targeting', which uses intrinsic carrier properties), and usually implies the existence of a high-affinity counterpart, the 'receptor', to which the ligand binds with specificity and which has a favourable biodistribution. The term receptor in this context should be understood to mean any well-defined

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structure that can act as a specific binding partner to a ligand.

Specific interactions provide the basis for biological regulation and 'natural' receptor–ligand combinations have provided the earliest source of reagents for drug targeting to tumours. Such endogenous receptor–ligand pairs have often been extensively characterised and their biological behaviour and physiology tend to be well understood. Hence, the ligand is often available in sufficient quantity from biological sources and the tools to make the recombinant protein are usually readily available. Furthermore, because they are already present within the body, their use gives less concern that an immune response could be triggered. However, the endogenous nature of the ligand often means the targeting ligand has to compete with the ubiquitous background level of the endogenous ligand, which might severely impede the efficiency of the targeting process.

Frequently, the receptor will be a structure on the cell surface that functions in a signal transduction capacity (e.g. the epidermal growth factor (EGF) receptor family; Ref. 64) or as a transporter [e.g. the transferrin receptor (TfR) (Ref. 65) and folate receptor (FR) (Ref. 66)], but other structures on the tumour cells or in the microenvironment could also provide a target. The receptor for the iron-binding transferrin protein (TfR, CD71) is typically overexpressed on proliferating cells and tumour cells (Refs 67, 68, 69), and its presence has also been linked to drug resistance (Ref. 70). The endogenous combination of transferrin and TfR has been used for the targeting of a large number of delivery systems (Refs 65, 71). Not only has TfR been targeted using its natural ligand, but also using antibodies such as OX-26 against TfR (Ref. 72). The antibody-binding site does not interfere with transferrin binding and thus is not affected by the relatively high levels of transferrin saturation experienced under physiological transferrin concentrations (Refs 73, 74). Another example is the combination of folate and FR, which is a potential tumour marker in ovarian, lung, colon and breast cancer (Refs 75, 76, 77, 78, 79). Hence, folate has been used extensively for the targeting of conjugates and particulate delivery systems (Ref. 80).

The number of naturally occurring receptor–ligand combinations with some tumour specificity is relatively limited. However, monoclonal antibodies and recombinant techniques have provided the tools that allow the identification and production of ligands de novo. The use of de novo-identified exogenous ligands potentially reduces interference from competing free ligand in the circulation, allows targeting of structures for which a biological targeting option does not currently exist, and also facilitates the tailoring of ligands to receptor subpopulations and the modulation of binding affinities. Several technologies are now available that provide huge libraries of potential ligands from which high-affinity ligands may be selected using panning and selection strategies. The libraries are based on recombinant libraries of antibody fragments (Ref. 81) and peptides (Ref. 82), or synthetic chemical libraries (e.g. peptides, aptamers; Ref. 83).

In considering the suitability of receptor–ligand combinations for targeting, one needs to consider the specificity of the receptor (i.e. the relative receptor numbers on the target versus normal tissue), as well as the absolute receptor number. For example, a receptor that is highly specific for the tumour but is present only in small absolute numbers might require extremely potent agents or carriers with a high drug load in order to be therapeutically efficacious. Hence, a high receptor density will normally be advantageous when using actively targeted systems. However, it is important to make the distinction between high receptor density and activity: high-level expression of the surface receptor does not guarantee that the cellular machinery for uptake is functional, as has been shown for EGF in A431 cells (Ref. 84). An ideal receptor would be highly specific for the targeted tumour cells (i.e. no expression in other parts of the body) and also have a high activity (i.e. the combination of high absolute receptor numbers and rapid internalisation/recycling of the receptor for subsequent internalisation cycles).

Another consideration is the accessibility of the receptors to the targeted delivery system – 'receptor' structures on tumour cells would only be accessible after extravasation, although in so-called mosaic blood vessels some of the tumour cell surface might be directly accessible (Ref. 21). Whereas high-affinity binding between receptor and ligand promotes specificity, there is a balance to be struck as very strong binding in the periphery of a tissue section might reduce further diffusional transport of targeted material towards the centre and thus could create a 'binding site barrier' (Ref. 85).

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A consequence of receptor-mediated internalisation is that the targeted delivery system will in general be taken up via receptor-mediated endocytosis and thus become enclosed in endosomes. In order for the drug to reach its site of action, and to avoid degradation by endosomal enzymes, subsequent escape into the cytoplasm will usually be required. For drugs such as doxorubicin, which can be non-covalently encapsulated in a carrier, diffusion will normally be sufficient to reach the site of action – the nucleus. In the case of conjugates, cleavage of the covalent link might first be required. In the case of drugs with less-favourable physicochemical properties, endosomal escape strategies are required. In fact, intracellular trafficking, in particular endosomal escape and transport to the nucleus, represent significant barriers for synthetic gene delivery systems that currently limit the overall efficiency of these gene therapy systems. By contrast, for some strategies, such as ADEPT (see below), it is not necessary or even desirable that the receptor is internalised (Ref. 86).

Targeting strategies

The transformation of normal cells into tumour cells is the result of the accumulation of genetic damage over extended periods of time (Ref. 87). A consequence of the wide range of changes that can contribute to this transformation is that the cancer phenotype is not narrowly defined but encompasses a broad array of changes even within one tumour type. One of the key problems in drug targeting to tumours is therefore that tumours are in fact not easily distinguishable from healthy cells by a single common feature that would facilitate drug targeting.

The additional specificity that drug targeting can provide over and above the pharmacodynamic specificity of the drug is based on the creation of a dose differential between tumour and healthy tissue. This dose differential can be achieved through the exploitation of various phenotypic differences between the diseased site and the rest of the body. These differentials exist at the organ and tissue level (e.g. EPR effect), as well as at the cellular and molecular level (e.g. high-level expression of the receptor). Tumour-targeting strategies combine the complementary specificity that the constituent elements of the antitumour medicine (drug, delivery system and control elements) provide at these levels. Targeting strategies can be loosely grouped into categories that define some key characteristics and provide a useful description of the underlying strategy (Table 1). However, it should be emphasised that boundaries between the systems are not rigid and that the same materials may be used to create delivery systems based on different strategies. For example, the water-soluble polymer glycolchitosan can be modified to make micelles, hydrogels, vesicles, nanoparticles and gene delivery systems depending on the specific physicochemistry (Refs 88, 89, 90, 91). Similar strategies have been used to create polymeric micelles from other materials (Ref. 92) and polymer–drug conjugates (Ref. 43).

The passive targeting of drug-loaded carrier aggregates using the EPR effect has been successfully used to deliver packaged LMW drugs of medium potency and specificity that act directly on the tumour cell. This type of system is exemplified by doxorubicin encapsulated in a particulate drug carrier (Ref. 62), or as part of a drug conjugate (Ref. 43). In this case, the dose differential afforded by the carrier (higher doses

Table 1. Summary of key properties of systems used in various tumour-targeting strategies[a]

Route of administration	Drug action	Delivery system	Targeting strategy modality	Administration
Local	Direct	Aggregate (vesicle, micelles or particles)	Passive	Single step
Systemic	Indirect	Molecular (stoichiometry or linker)	Active (endogenous or exogenous)	Multiple steps

[a] Strategies for the targeting of drug delivery systems can be loosely categorised according to these headings, although significant overlap might exist.

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can be administered and accumulate in the tumour) is combined with a sensitivity differential of the tumour cells to the drug to increase therapeutic specificity. Liposomal doxorubicin (also known as Caelyx®/Doxil® or Myocet®) and daunorubicin (DaunoXome®) is also the first example of the progression of a drug delivery system into wider oncological practice, with marketing authorisation in Europe and USA [n.b. the polymer conjugate SMANCS (copolymer styrene maleic acid-conjugated neocarzinostatin) has been in clinical use in Japan for longer; Ref. 93)]. Caelyx has received market authorisation in Europe (and USA) for the treatment of AIDS-related Kaposi's sarcoma, for metastatic breast cancer in patients with increased cardiac risk, and in ovarian cancer after failed first-line platinum-based chemotherapy. Myocet was initially licensed in Europe for the first-line treatment of metastatic breast cancer in combination with cyclophosphamide. The daunorubicin-containing DaunoXome liposomal formulation has an indication for the first-line cytotoxic therapy for advanced, HIV-related Kaposi's sarcoma. Doxorubicin as part of polymer–drug conjugates (PK1) has also been tested in the clinic (Ref. 94) and provides pharmacokinetic advantages that theoretically should translate into better efficacy. However, Phase II clinical trials of PK1 did not show activity at a level that was considered high enough to continue product development. The relatively LMW (~20 kDa) of PK1 might not have been sufficient to give the full EPR effect (Ref. 95).

In contrast to these strategies, active targeting provides the specificity that is crucial when the drug is highly potent but is not necessarily tumour specific, such as actively targeted antibody–toxin conjugates. Here, the additional specificity derived from the targeting becomes crucial, and the efficiency of delivery is a secondary concern (i.e. a few molecules will be sufficient). In order for this type of system to work, the accessibility, distribution and activity of the targeted structure/receptor is vital in creating the differential between tumour and healthy tissue. As a common feature for targeting of all tumour cells is not available, the suitability of a specific ligand–receptor combination for targeting of a specific tumour should ideally be determined on an individual basis.

Strategies that utilise agents that act indirectly (i.e. after an activation step) have the potential for being highly specific to tumour cells. An example of this approach is gene therapy where the drug (i.e. the gene) is acting indirectly rather than directly because the gene has to be transcribed/translated into the active protein by the target cell. Gene therapy strategies also provide an example of how specificity can be improved though a combination of differentials at different levels. Through targeting of vector delivery to the tumour, a gene dose differential is created. This transductional specificity can be combined with the transcriptional specificity (Ref. 96) through the use of tumour-specific promoters (e.g. telomerase; Ref. 97), which translates molecular differentials closely linked with tumour pathophysiology into a transgene dose differential. A combination of transductional and translational control is also being utilised in recombinant viral vectors that replicate conditionally only in tumour cells that suitably complement their reduced functionality (e.g. E1B deletion augmented by lack of functional p53; Ref. 98).

All of the above strategies only involve a one-step administration of the drug. As an alternative, it is possible to combine differentials through the introduction of additional multi-step administration steps. In the case of the directed enzyme prodrug therapies (DEPT), prodrug activation occurs through cleavage by a previously introduced exogenous enzyme (Ref. 99). The enzyme can be directed to the tumour using conjugation with a targeting ligand (antibody–ADEPT; Ref. 86), or by accumulation and precipitation of polymer-linked enzyme in the tumour (polymer–PDEPT; Ref. 100), or by directed transgene expression in cells in the tumour (GDEPT; Ref. 101). Each of the 'directing' steps can be optimised to maximise specificity. Because the drug and prodrug can diffuse into tumour cells, these approaches can target the tumour cells or the microenvironment. In contrast to the indirect GDEPT strategy, which requires gene expression, the ADEPT and PDEPT strategies deliver the enzyme directly and do not require internalisation of the targeted carrier or enzyme.

Discussion

Clinical relevance

Drug delivery has already arrived in clinical practice in the form of liposomes [e.g. Caelyx®/Doxil®; AmBisome (liposomes containing amphotericin B); Refs 102, 103], and polymer–drug conjugates of various types are in Phase II

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and limited Phase III studies. So far, all of these systems have used 'old' drugs as the payload for the targeting system. This is both conceptually and practically limiting because these compounds have limitations in their toxicity and in the development of drug resistance. It seems unlikely that such systems will make dramatic improvements to therapy in a general fashion. (However, in specific clinical situations, such systems have demonstrated their potential, for example in the use of intra-arterially administered polymer conjugate SMANCS in hepatocellular cancer; Ref. 93.) If one views these as 'proof of principle' or first-generation compounds, then it is more than likely that second-generation compounds will use novel agents as the payload. The 'ideal' candidate at this point might be a highly toxic molecule that has proven activity but has not made it to the clinic because of normal tissue toxicity or lack of solubility. Subsequent generations will attempt to deliver on the promise of novel therapeutic strategies that stem from the progress in the understanding of the molecular and genetic basis of cancer, in particular genetic therapies. Such therapies could have a major impact on the practices of oncology.

Research in progress and outstanding research questions

One of the key areas of interest of current research is to provide delivery systems that facilitate the clinical application of novel genetic therapies. At present, this problem is being approached from two different directions: viral vectors tend to be more efficient than nonviral systems but, because of (perceived) safety concerns, the pressure has been to reduce virus functionality to the barest minimum required for delivery; developers of synthetic (nonviral) gene delivery systems often endeavour to improve efficiency of these systems through the addition of elements that mimic the functionality of some viral proteins. One possible outcome of these developments could be a hybrid system that combines selected complementary elements of both these seemingly divergent strategies.

Regulation of biological processes is based on the re-use of a limited number of building blocks. Consequently, the excellent specificity of biological systems is defined through their spatiotemporal context and requires tight control on a cellular and molecular level. LMW drugs distribute throughout the body by diffusion according to their physicochemistry, which therefore offers little scope for the modulation of biodistribution. Thus, drugs with a more-subtle and well-defined mechanism of action might well require the development of a corresponding targeting and delivery technology that not only delivers to a target cell but also takes the cellular and molecular context into account.

Increasingly, there is recognition that, in order to optimise therapeutic success, it might be necessary to match the drug treatment with the molecular makeup of the cancer. The lack of a target common to most solid tumours means that an analogous individualisation may be necessary when targeting drugs and delivery systems to tumours. Although ligand libraries exist (e.g. in the form of peptide libraries or as recombinant phage libraries), there is currently no technology that would make the selection of appropriate ligands for an individual tumour practical.

The exploration of targeting strategies could be approached from either the materials/carrier viewpoint or from the tumour biology viewpoint. Although both approaches are valid and have contributed to clinical improvements, we would argue that, in the future, multidisciplinary teams with knowledge of both fields will be best placed to tackle the formidable challenge of targeted drug and gene delivery to the tumour.

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Further reading, resources and contacts

The intermolecular and interparticle forces behind drug carrier systems are explained in:
Israelachvili, J.N. (1992) Intermolecular and Surface Forces with Applications to Colloidal and Biological Systems (2nd edn), Academic Press

Further information on vesicles as drug delivery systems:
Torchilin, V.P. and Weissig, V., eds (2003) Liposomes: a Practical Approach (2nd edn), Oxford University Press
Uchegbu, I.F. (ed.) (2000) Synthetic surfactant vesicles: niosomes and other non-phospholipid vesicular systems. In Drug Targeting and Delivery, Harwood Academic

Further information on polymer drugs:
Maeda, H. et al. (2003) Polymer Drugs in the Clinical Stage: Advantages and Prospects, Kluwer Academic/Plenum Publishers

Further information on general drug delivery:
Advanced Drug Delivery reviews:
http://www.sciencedirect.com/science/journal/0169409X

Journal of Controlled Release:
http://www.sciencedirect.com/science/journal/01683659

Further information on polymer–drug conjugates and related areas:
Duncan, R. (2003) The dawning era of polymer therapeutics. Nat Rev Drug Discov 2, 347-360, PubMed:12750738
Maeda, H. (2001) The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzyme Regul 41, 189-207, PubMed:11384745

Further information on tumour barriers:
Jain, R.K. (1998) Delivery of molecular and cellular medicine to solid tumors. J Control Release 53, 49-67, PubMed:9741913

Further information on active targeting with ligands:
Allen, T.M. (2002) Ligand-targeted therapeutics in anticancer therapy. Nat Rev Cancer 2, 750-763, PubMed:12360278

Features associated with this article

Figures

Figure 1. Transport in tumours compared with normal tissue.
Figure 2. Targeted delivery systems: carrier-based systems and drug conjugates.

Table

Table 1. Summary of key properties of systems used in various tumour-targeting strategies.

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