available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.ejconline.com

Delivering cancer stem cell therapies – A role for nanomedicines?

*Andreas G. Schätzlein**

CRUK Centre for Oncology and Applied Pharmacology, Cancer Research UK Beatson Laboratories, University of Glasgow, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, UK

ARTICLE INFO

Article history:
Received 23 January 2006
Accepted 23 January 2006
Available online 8 May 2006

Keywords:
Nanomedicines
Drug delivery
Vesicles
Micelles
Gene delivery
Drug resistance
CD44
Ligands
Active targeting
Drug conjugates
ABC transporter family

ABSTRACT

Cancer stem cells (CSCs), i.e. cancer cells that can self-renew, constitute only a minority of the cells of a tumour, but, because of their ability to initiate and repopulate tumours, failure to control CSCs can potentially lead to tumour re-growth, even though the bulk tumour may have been treated successfully. Nanomedicines improve spatio-temporal control over drug kinetics and distribution, thus opening the prospect of safer and more specific therapies to address the challenges posed by CSCs. In particular, these systems have the potential to facilitate CSC-aware therapy by overcoming resistance to conventional cytotoxic drugs and by targeting novel therapies to the tumour and CSC-marker positive cells. This review examines the implications of the CSC paradigm specifically for the development of nanomedicines, i.e. therapies based on macromolecules or supramolecular aggregates.
© 2006 Elsevier Ltd. All rights reserved.

1. Introduction

The ideas behind the cancer stem cell (CSC) hypothesis are not new and have evolved over last 150 years.[1] Nonetheless, the recent reports of side-populations of cells with stem-cell-like properties in solid tumours of the breast and brain[2,3] have led to these concepts being re-examined more widely. Importantly, the CSC paradigm potentially explains and links together a number of clinical observations with relevance to therapeutic outcome. While we have only just started to appreciate the potential implications, it is probably not too early to examine what bearing these ideas might have on therapeutic strategies for cancer therapy. This review will, however, not examine the implications for therapeutic small molecules[4] but will consider the implications of the cancer stem cell paradigm for development of nanomedicines, i.e. therapies based on macromolecules or supramolecular aggregates.

The modern paradigm of a anti-cancer drug lead is a small molecule that interacts strongly with well-defined, unique molecular targets with a defined role in the pathogenesis of cancer, with the therapeutic index largely depending on the specificity of the interaction between drug candidate and target. This approach has delivered interesting new therapies, but it may have limitations, as specificity of molecular interactions in the cell is not based on structure alone but is also defined by molecular and cellular context. Some molecules will have different, sometimes opposing, functions/effects

* Tel.: +44 141 330 4354; fax: +44 141 330 4127.
E-mail address: A.Schatzlein@beatson.gla.ac.uk.
0959-8049/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ejca.2006.01.044

depending on the molecular context. Furthermore, the structural variety of biological molecules is frequently based on the use of a theme and variations, i.e. large structurally related super-families of molecules that share many features. However, current strategies for therapies using small molecule drugs offer only very limited control over the cellular and molecular context of drug action other than the effects of physical chemistry on drug distribution and pharmacokinetics.

Nanomedicines are being developed with a view to offering improved spatio-temporal control over drug kinetics and distribution; thus opening the prospect of safer and more specific therapies.

2. Nanomedicines

Nanomedicines are a recent off-shoot of the application of nanotechnology to medical and pharmaceutical challenges, but have in fact been around for much longer in the guise of drug delivery systems.

Nanomedicines are based on technologies and materials engineered and developed to operate on the nanometre scale and have novel properties that are of direct medical benefit or, more typically, which enhance and enable other therapeutic strategies. Nanomedicines that facilitate uptake and transport of therapeutically active molecules ('delivery systems') tend to be based on supramolecular assemblies of drug and functional carrier materials.

The use of nanomedicines facilitates the creation of dose differentials between the site of disease and the rest of the body, thus maximising therapeutic effect while minimising non-specific side-effects. Their development is based on a number of key concepts that govern the interaction of these systems with the body, as explained here.

Pharmacokinetics and biodistribution of a drug are predominantly governed by its physical chemistry, e.g. the size of a molecule, its polarity, number and nature of hydrophobic and hydrophilic residues, etc. These proprieties can sometimes be adjusted without loss of activity using medicinal chemistry. Alternatively, nanoscaled biomaterials can be used to encapsulate the drug molecule and thus 'hide' unfavourable interactions between drug and the body, effectively replacing them with the properties of the nanomedicine (drug ↔ body replaced by [drug]-nanomedicine ↔ body). This is particularly important during the absorption/distribution process, whereas the subsequent release of the drug at the target site reverses this process (Fig. 1).

This can be achieved using two distinct approaches, i.e. *packing* (the use of nanoscaled containers that carry multiple drug molecules typically within) and *tagging* (covalent conjugation of the drug molecules with carrier and targeting moieties that override drug physicochemistry and thus determine pharmacokinetics (PK) and biodistribution).[5]

The packing of drug molecules normally involves non-covalent association with, or encapsulation within, a carrier. These carriers function as nanometre-scaled containers for the drug and are generally created from smaller monomers that self-aggregate to form particles with sizes of several 10–100 s of nm. Examples include liposomes and similar vesicles, micelles, or solid nanoparticles. These types of colloidal, or particulate, carriers typically have a relatively high drug loading, i.e. they normally carry a large number of drug molecules per particle.[5]

Alternatively, drug molecules can be 'tagged' to alter their properties in order to target them to tumours. This normally involves the covalent conjugation of a targeting moiety to each individual drug molecule or the coupling of the drug molecules to a multivalent carrier molecule that can then be targeted. An example of the former strategy is the targeting of potent toxins coupled to an antibody, while the latter approach is exemplified by the use of polymer–drug conjugates, where the polymer serves as the carrier molecule.[5] These types of nanomedicines, also referred to as 'macromolecular' (molecular weights (MWs) in the order of 10^3–10^4 Da),

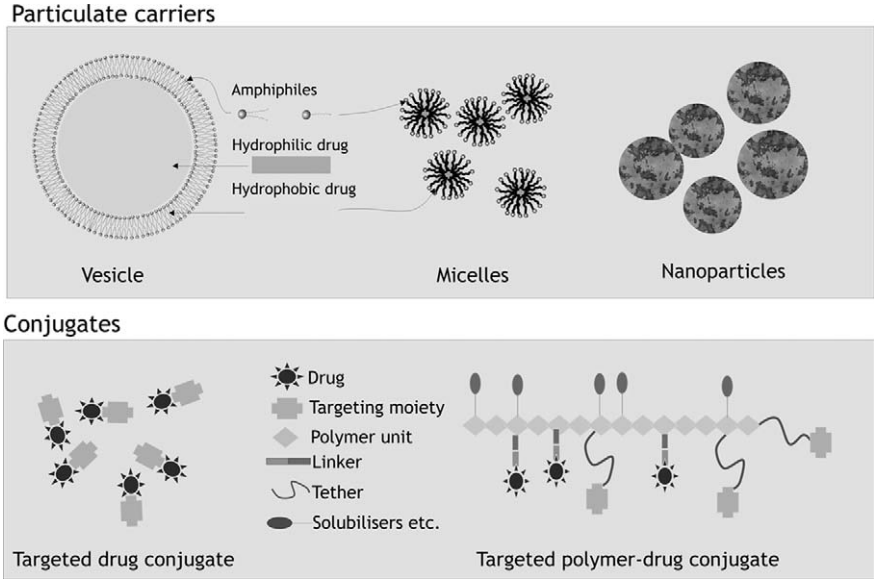

Fig. 1 – Nanomedicines deliver drugs either by 'packing', i.e. non-covalent aggregates of carrier molecules encapsulate multiple drug molecules in carriers, or by 'tagging' using covalent coupling to a targeting ligand or a carrier polymer.

are larger than typical 'small molecule' type of drugs (MW $\sim 10^2$ Da), but significantly smaller than the particulate nanomedicines (see Fig. 1).

Drug molecules associated with a delivery system need to be released in an active form at the target site in order to be able to induce the desired therapeutic effects. Packing, i.e. the non-covalent encapsulation of drugs in the particulate carrier, does not chemically modify the drug molecule. The drug is, in general, released gradually through a slow exchange/leakage from the carrier or more rapidly once the nanomedicine is broken up, e.g. inside the cell.

Covalent linking of drug and targeting/carrier molecule ('tagging') creates a new chemical entity with potentially modified activity. The use of cleavable linkers for drug conjugation not only facilitates drug release at the target site, thus restoring the original drug molecule, but can also contribute to the overall specificity of the nanomedicine if release is triggered *selectively* at the target site. Examples of the use of this strategy are the use of peptide linkers that are cleaved by enzymes enriched in the tumour, e.g. matrix metalloproteinases (MMPs) or endosomal peptidases. In the antibody-directed enzyme-prodrug therapy (ADEPT) approach an exogenous enzyme is targeted to the tumour to then specifically release the active drug in a two-step procedure.

In order to harness the potential of nanomedicines, the biology of CSCs and their role in tumour development needs to be understood in detail to allow tailoring of nanomedicines for the development of CSC-aware therapeutic strategies.

3. Nanomedicine strategies for cancer stem cell therapy

The expression 'cancer stem cell', rather than describing a well-defined cell type or origin currently primarily conveys the concept that, among the heterogeneous cell populations that makes up the tumour, there exist cells that share with stem cells a number of key properties. Specifically, such cells have the ability for self-renewal, while at the same time producing daughter cells that have significant proliferation potential and that form part of a differentiation dependent hierarchy.

The ability for *self-renewal* implies the ability of cells to maintain a pool of relatively less differentiated stem cells. These can divide repeatedly in a cycle that creates a daughter cell with high proliferation potential and a second daughter that retains the properties and potential of the original stem cell. Cancer stem cells are transformed cells with the capability for self-renewal, also known as tumour-initiating cells (TIC) as it is this minority of cells that upon transplantation give rise to a larger population of proliferating but non-tumourigenic cells that form the bulk of the tumour as well as more TICs.[2] This process includes *proliferation* and *differentiation* and resembles organ formation during embryonic development as well as the maintenance of high turnover tissues through adult stem cells. The capacity for self-renewal and the ability to proliferate are thus not necessarily linked; in fact, the low proportion of TIC in tumours suggests that proliferation is predominantly restricted to the non-tumourigenic population.

Many important questions, such as to what extent cancer stem cells are involved in the pathology of all human solid tumours, or, whether cancer stem cells originate from mutated physiological stem cells or from more differentiated cells that reactivate stem cell functionality, currently remain unanswered. Nevertheless, based on our understanding of the physiology of normal somatic stem cells and the more advanced understanding of the role of stem cells in haematological cancers[6] a picture emerges that permits consideration of the implications of CSC biology for therapeutic strategies.

4. Developing CSC-directed nanomedicines

The CSC paradigm forces us to re-examine the way potential drug leads are being identified and developed. One of the key challenges for the development of CSC-directed therapies lies in the limitation of current model systems.

Efficacy in pre-clinical models is normally assessed as a function of the degree of remission or growth delay induced by a treatment. This approach only takes into account reduction of tumour bulk and does not differentiate between the tumourigenic cells that can repopulate the tumour and the population of non-tumourigenic but proliferating cells which represent the bulk of the tumour. Therefore, we have to question the utility of current models that largely measure therapeutic impact as a function of reduction in bulk tumour volume. What will be needed are animal models with well-defined cell compartment and highly sensitive assays capable of monitoring even small subpopulations.

It is unclear at this point to what extent the microenvironment may play a role in maintaining the self-renewal capacity of cancer stem cells. In the case of somatic stem cells the existence of stem cell niches and the importance of even subtle changes in the microenvironment have been established.[7] There is evidence demonstrating the importance of microenvironment for tumour growth and development[8] and if CSCs are sensitive to the histological context this could pose a significant challenge for the development of tumour models.

Cancer stem cells, independent of whether they may have re-acquired stem cell properties or are in fact derived from normal stem cells, are likely to share some of these characteristics with normal stem cells.

In order to allow long-term self-renewal and provide a pool of pluripotent cells that can replenish an organ, normal adult stem cells have an increased resistance to toxins/drugs through the expression of a number of ABC-type transporters, the ability for extensive DNA repair and an increased resistance to apoptosis. As the main proliferation occurs largely in the derived progenitors the stem cell pool is also largely quiescent. Furthermore, the balance of pro-apoptotic and anti-apoptotic activities tends to be biased towards long-term survival.

The idea that CSCs may share some of these protective molecular mechanisms with normal stem cells would help to explain the clinical observation of apparent initial therapeutic success as judged by reduction in tumour bulk, which is eventually followed by re-growth of the tumour.

In fact, a mathematical model based on the presence of resistant CSCs accurately describes the kinetics of this process in Chronic myelogenons leukaemia (CML).[6]

Principally, there are therefore two strategies to be considered for CSC-aware cancer therapies: exposure of CSCs to

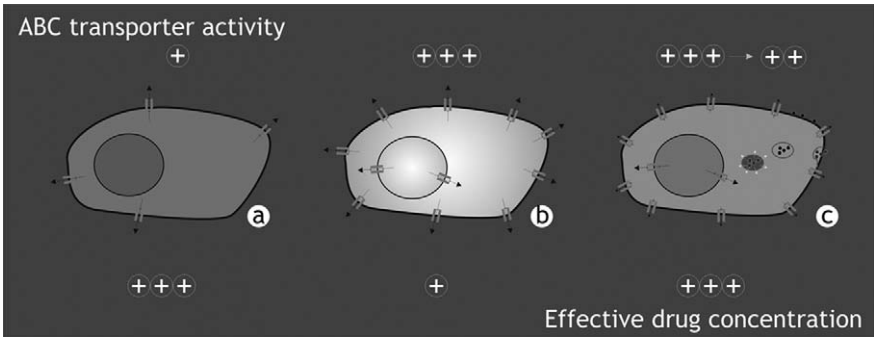

Fig. 2 – Small molecule drugs enter cells by diffusion across the cell membrane. (a) In cells with low activity of ABC type drug transporters this leads to similar extracellular and intracellular drug concentrations, with the drug acting via the default mechanism, e.g. in nucleus. (b) In cells with high levels of ABC transporter activity the intracellular concentration of drugs that are substrate for the transporter is lowered efficiently and is insufficient to be efficacious. (c) The use of nanomedicines for the delivery of transporter substrate drugs can overcome some of the resistance by changing the site of activity/ mechanism (e.g. cell surface versus intracellular) and by a change in route of uptake (typically via endocytosis). In addition some systems have also been shown to reduce overall transporter activity.

sufficiently high levels of conventional cytotoxic agents and the development of novel therapeutic agents that are *targeted* to CSCs.

The key limitation for increasing the exposure of CSCs to higher levels of cytotoxic drugs lies in their non-specific nature and the fact that they are already used at concentrations just below the levels at which dose-limiting toxicity is expected. Therefore, only approaches that achieve higher exposure as well as improved specificity, e.g. by targeting, can potentially realise this strategy. Alternatively, increased exposure can also be achieved at the cellular level if transport-related resistance mechanisms can be overcome.

4.1. *Overcoming drug resistance*

One of the key mechanisms for drug resistance has been linked to the activity of transporters that pump substrate drugs out of the cell thus reducing the effective drug concentration within the cell. The ABC-type transporters, specifically pgp/MDR1, have been the focus of much research, and nanomedicine systems have been shown to be able to overcome this type of resistance in laboratory models. The clinical relevance of transporters for intrinsic or acquired resistance of tumours is less clear and clinical study designs may have to be reconsidered in the light of the existence of CSCs in order to be able to answer this question.

The ABC transporters are a super-family of ATP-cassette drug/toxin transporters that interact with a wide range of hydrophobic and hydrophilic substrates (Table 1). Members of this transporter family are expressed at high levels in stem cells, but have also been linked to the resistance of tumours to chemotherapy.

The ability of transporters such as p-gp or MRP1 to reduce the intracellular concentration of substrate compounds has in fact been used to identify subpopulation of cells. These so-called side-populations overlap to some degree with the stem cell population and are characterised by a reduced concentration of intracellular fluorescent dyes that are substrate for the transporters. Consequently, these cells appear ‘dull’, i.e. they can be enriched for by flow cytometry based on the reduced fluorescence compared with the main population.

The ABC transporters constantly remove the substrate drugs from the cytosol, thus effectively reducing the intracellular drug concentration, but other transporter-related effects, such as altered intracellular distribution, have also been implicated in resistance.[10]

Table 1 – The ABC transporters[9]

Gene	Protein/alias	Chemotherapeutic drugs effluxed by transporter	Other drugs and substrates
ABCA2	ABCA2	Estramustine	–
ABCB1	PGP/MDR1	Colchicine, doxorubicin, etoposide, vinblastine, paditarel	Digoxin, saquinivir
ABCC1	MRP1	Doxorubicin, daunorubicin, vincristine, etoposide, colchicine, camptothecins, methotrexate	Rhodamine
ABCC2	MRP2	Vinblastine, cisplatin, doxorubicin, methotrexate	Sulfinpyrazone
ABCC3	MRP3	Methotrexate, etoposide	–
ABCC4	MRP4	6-Mercaptopurine, 6-thioguanine and metabolites, methotrexate	PMEA, cAMP, cGMP
ABCC5	MRP5	6-Mercaptopurine, 6-thioguanine and metabolites	PMEA, cAMP, cGMP
ABCC6	MRP6	Etoposide	–
ABCC11	MRP8	5-Fluorouracil	PMEA, cAMP, cGMP
ABCG2	MXR/BCRP	Mitoxantrone, topotecan, doxorubicin, daunorubicin, irinotecan, imatinib, melhotrexate	Pheophorbide A, Hoechst 33342, rhodamine

Based on the link between drug transporter activity and resistance demonstrated in the laboratory, small molecule inhibitors of drug transporters, mainly of p-gp, have been developed. The second and third generation of these compounds have shown some promise in pre-clinical evaluation, but, despite a demonstrated effect on the target ABCB1 in tumours, were only of limited clinical impact.[9]

Nanomedicines and drug delivery systems have previously been utilised to address the problem of drug resistance and may offer some inherent advantage over small-molecule-based ABC transport inhibitors as they can interfere with resistance mechanisms at different levels (see Fig. 2).

While small molecule drugs will normally enter the cell through diffusion, this is not generally the case for nanomedicines. Whether the drugs are packed, i.e. encapsulated in some form of particulate carrier, or tagged, that is covalently linked to a carrier or targeting moiety, they are taken up by endocytotic processes rather than by diffusion. As a consequence, they initially interact with the cell membrane and are then taken up in endocytotic vesicles and are thus not immediately accessible to the drug transporters in the cellular membrane.

Doxorubicin linked to a hydroxypropylmethacrylamide (HPMA) polymer backbone with peptide linkers that release the drug in the endosome were shown to circumvent at least some of the efflux-pump-related resistance,[11] thus demonstrating the potential of polymer drug conjugates.[12] Particulate carrier systems in which the drug is packed have also been reported to have increased activity in drug-resistant cells compared with the free drug. For example, Kabanov and colleagues have used micellar aggregates of the block-co-polymer Pluronic as carrier for anti-cancer drugs.[13] Interestingly, the polymer not only has advantages in formulating doxorubicin, but also sensitise resistant cells, particularly against anthracyclins. The polymer was active against various resistance mechanisms at different levels, such as inhibition of ABC-type transporters, abolishing drug sequestration in acidic vesicles, and inhibition of the glutathione S-transferase detoxification system. These activities have been proposed to be linked to a polymer-dependent ATP depletion.[14,15]

Cytotoxic drugs such as the anthracyclins do not have a single molecular target, but affect the cell at different levels. Consequently, the modification of cellular uptake and intracellular distribution of drug can also result in modulation of the pharmacology. Zokes and colleagues demonstrated in 1982 that doxorubicin coupled covalently to microspheres was active despite the lack of intracellular free drug.[16] Clearly, this shift from intracellular/nuclear target to membrane interaction would bypass many of resistance mechanisms[10] but also has the potential to modulate pharmacology: doxorubicin treatment of cancer cells induces both, a pro-apoptotic and a counter-acting anti-apoptotic signal. In contrast, doxorubicin given in the Pluronic micellar system does not induce an anti-apoptotic response and is therefore comparatively more efficient at inducing cell death.[17] This type of approach may therefore be ideally suited to the therapy of CSCs, where, similar to normal stem cells, this balance may be biased towards anti-apoptotic signals to ensure long-term cell survival.

Clearly, not all tumour cells that express the ABC transporters are stem cells and not all transporters are necessarily expressed in cancer stem cells. The observation that many of the stem cells found in the side-population express ABCG2 rather than the ABCB1 p-gp/MDR1 transporter, that have been the focus of much of the inhibitor development, may contribute to the lack of clinical impact:[9] an inhibitor that efficiently inhibits p-gp in the majority of tumour cells but is ineffective against a stem cell population is likely to have no long-term clinical benefit. At present the role of the various mechanisms and transporters in CSCs are still emerging.[18] Nevertheless, looking at this challenge with the paradigm of cancer stem cells in mind may allow a new understanding/re-evaluation of the underlying biology.

Novel therapies can be targeted to CSCs either at the molecular level or at the cellular level. Targeting at the molecular level will require discovery and validation of CSC-specific therapeutic targets followed by identification and development of the corresponding leads. As an alternative strategy targeting of CSCs at the cellular level can exploit active targeting of nanomedicines to increase the therapeutic index of current therapies or facilitate targeted delivery of novel therapies such as gene-based approaches.

4.2. *Targeting nanomedicines*

Nanomedicines engineered to target tumours or cancer cells effectively create a dose differential, i.e. they maximise drug exposure at the diseased site and minimise drug levels in healthy tissues. Such targeted nanomedicines thus increase the specificity of a therapeutic strategy over and above that achievable with the drug alone.[5,19]

Targeting strategies fall into two groups, 'active' or 'passive'. Active targeting exploits the molecular interaction/binding of a specific targeting ligand to a defined molecule that, in the broadest sense, acts as 'receptor'. By contrast, passive targeting does not rely on the presence of a specific 'receptor', but exploits haemodynamic changes linked to the development of the blood supply in many solid tumours: the leaky tumour neovasculature can act as a sieve that allows extravasation and subsequent accumulation of particulate and macromolecular nanomedicines based on the so-called enhanced permeability and retention (EPR) effect.[20] Passive targeting strategies thus are potentially more broadly applicable, i.e. in various tumour types, but also relatively less specific and reliant on a tumour sufficiently large to induce angiogenesis.

Conversely, active targeting strategies have the potential to be more specific and actually target individual cells based on the presence of a suitable receptor. Ligands for active targeting can be derived from endogenous, physiological receptor–ligand combinations, such as transferrin–transferring receptor or folate–folate receptor, but can also extend to include other structures on the tumour cells or in the microenvironment where a ligand needs to be discovered de novo, e.g. by induction of antibodies or based on screening of recombinant or synthetic libraries. However, so far no universal 'cancer-specific' receptor has been identified and is in fact unlikely to exist, thus making it necessary to match targeting strategy to tumour/cell phenotype.

The concept of targeting always exploits phenotypical differentials, e.g. specific receptors, between the tumour/target

cell and the rest of the body that is then translated into a dose differential between target and off-target sites. It is now important to identify differentials that could be exploited to target the CSCs or to distinguish between CSCs and normal somatic stem cells. One of the most obvious starting points for this is based on the way CSCs are generally identified.

The identification and selection of stem cells and CSCs relies heavily on flow cytometry and fluorescently labelled antibodies, i.e. the combination of presence of markers of 'stem'-ness and absence of differentiation markers. An overview of markers used for the identification of various normal stem cell populations can be found at: http://stemcells.nih.gov/info/scireport/appendixe.asp#eii.

The CSCs, whether they are derived from stem cell typed populations or from more differentiated cells that acquire stem cell like properties, may share some of these markers with normal cells. Markers found on CSC in the clinic to date suggest that this may indeed be the case.

For solid tumours of the breast, the combination of CD44/$CD24^{low/-}$ has been shown to be a marker combination found on the majority of CSCs (eight out of nine),[2] but additional markers may emerge.[21]

CSCs were also successfully isolated from a number of different brain tumours based on their expression of the marker CD133,[22] a membrane-spanning glycoprotein expressed on the surface of many somatic stem cells, e.g. haematopoietic,[23] muscle[24] or prostate epithelium,[25] in melanoma CD20 has recently identified as marker of tumourigenic subpopulations.[26]

Interestingly, CD44 has been considered as a potential 'receptor' for tumour targeting even before its identification as an important marker of CSC in breast cancer was known. The CD44 receptor has been implied in the pathogenesis of cancers in various roles.[27] The natural ligand for CD44, the polysaccharide hyaluronan, has properties that make it particularly interesting as the basis for biomaterials and drug delivery systems.[28,29] More recently, it was shown that hyaluronan-based oligomers can also be used to advantage for the targeting of nanomedicines to cancer cell lines that express the CD44 receptor.[30,31]

One of the key challenges is the transfer of such systems into the much more complex in vivo situation, where the presence of CD44 and other hyaluronan-binding proteins, such as Receptor for hyaluronan-mediated motility (RHAMM), and the competition with soluble CD44 receptor or hyaluronan will potentially interfere with targeting. Encouragingly, targeting of doxorubicin- and mitomycin C-liposomes with hyaluronan has also been shown to be feasible in subcutaneous xenografts and experimental metastases.[32,33] In our own laboratory, we have are developing synthetic gene medicines[34] and are currently developing CD44-targeted systems.[35]

The combination of CD44 and hyaluronan is an example of the use of an endogenous ligand for targeting. Alternatively, the technology for the targeting of nanomedicines with antibodies is well developed and would potentially allow a relatively rapid translation from other diagnostic antibodies (flow cytometric identification) to targeting of therapeutics.

However, while systems targeted to a potential CSC marker show promise, the lack of model systems means that it is currently very difficult to test with rigor whether this approach would also target CSCs within a tumour. The CSC may not necessarily express the putative 'receptor' at a high level and in a state that allows efficient internalisation. Furthermore, the histological organisation of CSC in a tumour is unknown and may well affect the ease of access: the atypical morphology of neovasculature leads to haemodynamic irregularities that mean that not all regions in the tumour are well perfused and transport in the interstitium is often very limited.[5]

Targeting of CSC within the context of a tumour potentially allows the use of both passive and active targeting strategies, but also brings the challenge of barriers to distribution within the tumour. An alternative therapeutic scenario is one that would take advantage of the clinical observation that tumours frequently respond well to the initially conventional therapy: Initial debulking of the tumour would be followed by a second phase of treatment that would set out to seek and destroy residual CSCs that could potentially re-populate the tumour. In this case the challenge lies less in access to the target cells, but more in the fact that in the absence of a well-developed tumour vasculature active targeting alone needs to be sufficiently specific and efficient to deliver the nanomedicines to the target cells.

5. Conclusion

Nanomedicines offer improved spatio-temporal control over drug kinetics and distribution, thus opening the prospect of safer and more specific therapies. Despite the significant technical challenges in the development of CSC-aware nanomedicines, not least related to the lack of appropriate model systems, these systems may be able to make some important contributions. In particular, these systems have the potential to facilitate therapy by overcoming resistance to conventional cytotoxic drugs and by targeting novel therapies to the tumour and CSC-marker positive cells. It is likely that therapies that have the required level of specificity will be based on the ability to exploit differentials at various levels, e.g. the combination of molecular specificity of drug–target interaction or transcriptional targeting together with the additional specificity derived from delivery in nanomedicines.

Clearly the development of CSC-aware therapeutic strategies represents an enormous challenge. The role of CSCs in the development and progression of tumours as well as a potential reason for therapy failure remains uncertain. Nonetheless, the potential of this paradigm shift to have an impact on the way cancer therapies are being developed is far-reaching and thus warrants further exploration.

Conflict of interest statement

None declared.

REFERENCES

1. Huntly BJ, Gilliland DG. Leukaemia stem cells and the evolution of cancer-stem-cell research. *Nat Rev Cancer* 2005;**5**(4):311–21.

2. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* 2003;**100**(7):3983–8.
3. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003;**63**(18):5821–8.
4. Al-Hajj M, Becker MW, Wicha M, Weissman I, Clarke MF. Therapeutic implications of cancer stem cells. *Curr Opin Genet Dev* 2004;**14**(1):43–7.
5. Schätzlein AG. Tumour targeted drug and gene delivery: principles and concepts. *Expert Rev Mol Med* 2004;**6**(19):1–17.
6. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. *Nature* 2005;**435**(7046):1267–70.
7. Powell K. Stem-cell niches It's the ecology, stupid! *Nature* 2005;**435**(7040):268.
8. Bissell MJ, Radisky D. Putting tumours in context. *Nat Rev Cancer* 2001;**1**(1):46–54.
9. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005;**5**(4):275–84.
10. Larsen AK, Escargueil AE, Skladanowski A. Resistance mechanisms associated with altered intracellular distribution of anticancer agents. *Pharmacol Therapeut* 2000;**85**(3):217–29.
11. Minko T, Kopeckova P, Pozharov V, Kopecek J. HPMA copolymer bound adriamycin overcomes MDR1 gene encoded resistance in a human ovarian carcinoma cell line. *J Control Release* 1998;**54**(2):223–33.
12. Duncan R. The dawning era of polymer therapeutics. *Nature Rev Drug Discovery* 2003;**2**(5):347–60.
13. Venne A, Li S, Mandeville R, Kabanov A, Alakhov V. Hypersensitizing effect of pluronic L61 on cytotoxic activity, transport, and subcellular distribution of doxorubicin in multiple drug-resistant cells. *Cancer Res* 1996;**56**(16):3626–9.
14. Kabanov AV, Batrakova EV, Alakhov VY. An essential relationship between ATP depletion and chemosensitizing activity of Pluronic block copolymers. *J Control Release* 2003;**91**(1–):75–83.
15. Kabanov AV, Batrakova EV, Alakhov VY. Pluronic block copolymers for overcoming drug resistance in cancer. *Adv Drug Deliv Rev* 2002;**54**(5):759–79.
16. Tokes ZA, Rogers KE, Rembaum A. Synthesis of adriamycin-coupled polyglutaraldehyde microspheres and evaluation of their cytostatic activity. *Proc Natl Acad Sci USA* 1982;**79**(6):2026–30.
17. Minko T, Batrakova EV, Li S, et al. Pluronic block copolymers alter apoptotic signal transduction of doxorubicin in drug-resistant cancer cells. *J Control Release* 2005;**105**(3):269–78.
18. Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. *Cancer Res* 2005;**65**(14):6207–19.
19. Schätzlein AG. Targeting of synthetic gene delivery systems. *J Biomed Biotechnol* 2003;**2003**(2):149–58.
20. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release* 2000;**65**(1–2):271–84.
21. Ponti D, Costa A, Zaffaroni N, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer Res* 2005;**65**(13):5506–11.
22. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. *Nature* 2004;**432**(7015):396–401.
23. Yin AH, Miraglia S, Zanjani ED, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 1997;**90**(12):5002–12.
24. Alessandri G, Pagano S, Bez A, et al. Isolation and culture of human muscle-derived stem cells able to differentiate into myogenic and neurogenic cell lineages. *Lancet* 2004;**364**(9448):1872–83.
25. Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, Collins AT. CD133, a novel marker for human prostatic epithelial stem cells. *J Cell Sci* 2004;**117**(pt 16):3539–45.
26. Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005;**65**(20):9328–37.
27. Toole BP. Hyaluronan: from extracellular glue to pericellular cue. *Nat Rev Cancer* 2004;**4**(7):528–39.
28. Larsen NE, Balzas EA. Drug delivery systems using hyaluronan and its derivatives. *Adv Drug Del Rev* 1991;**7**(2):279–93.
29. Drobnik J. Hyaluronan in drug delivery. *Adv Drug Del Rev* 1991;**7**(2):295–308.
30. Coradini D, Pellizzaro C, Miglierini G, Daidone MG, Perbellini A. Hyaluronic acid as drug delivery for sodium butyrate: improvement of the anti-proliferative activity on a breast-cancer cell line. *Int J Cancer* 1999;**81**(3):411–6.
31. Eliaz RE, Szoka Jr FC. Liposome-encapsulated doxorubicin targeted to CD44: a strategy to kill CD44-overexpressing tumor cells. *Cancer Res* 2001;**61**(6):2592–601.
32. Peer D, Margalit R. Loading mitomycin C inside long circulating hyaluronan targeted nano-liposomes increases its antitumor activity in three mice tumor models. *Int J Cancer* 2004;**108**(5):780–9.
33. Peer D, Margalit R. Tumor-targeted hyaluronan nanoliposomes increase the antitumor activity of liposomal Doxorubicin in syngeneic and human xenograft mouse tumor models. *Neoplasia* 2004;**6**(4):343–53.
34. Schätzlein AG. Synthetic anti-cancer gene medicine exploits intrinsic anti-tumour activity of cationic vector to cure established tumours. *Cancer Res* 2005;**65**(18):1.
35. Schätzlein AG. Tumour targeting of gene expression using hyaluronic acid–polypropylenimine dendrimer conjugates. In: *Proceedings of the 30th annual meeting and exposition of the controlled release society*, 19–23 July 2003. Glasgow (UK): Controlled Release Society; 2003, p. 635.