Published in final edited form as:
Nanomedicine (Lond). 2012 October ; 7(10): 1467–1470. doi:10.2217/nnm.12.115.

Biocompatible nanopolymers: the next generation of breast cancer treatment?

Julia Y Ljubimova and
Nanomedicine Research Center, Department of Neurosurgery, Samuel Oschin Comprehencive Cancer Center, Cedars-Sinai Medical Center, 8631 West Third Street, Suite 800E, Los Angeles, CA 90048, USA, Tel.: +1 310 423 0834, Fax: +1 310 423 0810, ljubimovaj@cshs.org

Eggehard Holler
Nanomedicine Research Center, Department of Neurosurgery, Cedars Sinai Medical Center, 110 N George Burns Road, Davis 2100, Los Angeles, CA 90048, USA

Keywords

biomarkers; breast cancer; nanodrugs; nanopolymers; personalized medicine; polymalic acid

"*Among modern drug formulations, nanopolymers are highly promising vehicles for multi-targeting and can provide molecular combination therapy and, thus, personalized therapy designed to treat individual tumors with specific marker expression profiles.*"

Background

Breast cancer is the number one cancer in the world among gynecological cancers. Breast cancer comprises heterogeneous tumors even among the three major groups classified by endocrine hormones and HER^+ biomarker expression: estrogen receptor positive and progesterone receptor positive, $HER2^+$ and triple negative: estrogen receptor negative, progesterone receptor negative and $HER2^-$ with its substantial overlap with basal-type and *BRCA1*-related tumors. Triple-negative tumors are often positive for EGF receptor-1. The individual patient treatment for these types of cancers is particularly important, as their therapy response, outcomes and management are significantly different. Currently, the success of treatment of these tumors with chemotherapy and biological agents, such as Herceptin®/trastuzumab for $HER2^+$ tumors, is limited, especially for triple-negative cancers. This necessitates the development of new treatment modalities, among which nanopolymers are gaining increased attention.

Correspondence to: Julia Y Ljubimova.

Financial & competing interests disclosure
The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

Nanopolymers: a molecular basis for personalized medicine

What are ‘nanopolymers’? The term ‘nanopolymer’ is ill defined and needs revision. We use the definition of nanopolymer in the word’s original sense, meaning a single polymer molecule in the nanoscale range. Micelles and other selfassembled or aggregated forms are termed ‘nanopolymer composites’. Nanopolymers, unlike most composites, cannot dissociate in solutions.

Advanced drug nanopolymers are polymeric platforms, which are chemically bound to a variety of functional groups/moieties. Functional groups are attached by controlled chemical reactions at pendant carboxyl, hydroxyl or amino groups of the polymer backbone. The backbone itself may contain all carbon or be a composition of carbon, oxygen and/or nitrogen atoms, depending on the chemical nature of monomers employed for polymer synthesis. Natural backbones contain oxygen and/or nitrogen that render them, in most cases, biodegradable. Recent interest in nanopolymers shifted from synthetic all-carbon backbones such as that of *N*-(2-hydroxypropyl)methacrylamide (HPMA) to natural-derived polyethers, polyacetals, polyesters or polyamides. Similar rules apply to dendrimers, except that they assemble into sphere-like multibranched molecules whose interior is difficult to access by solvent and enzymes for degradation. Examples of moieties that can be covalently attached to nanopolymers include:

- Peptides or proteins to target molecular tumor markers;
- Chemotherapeutic drugs and/or oligonucleotides usually in the form of prodrugs that are activated inside tumor cells through cleavage of linkers;
- Membranolytic groups that are activated in response to acidic pH in mature endosomes/ lysosomes;
- Polyethylene glycol protecting against degradation and scavenging;
- Groups for tumor imaging; all moieties may be present on one polymer.

Nanopolymers can be linear or branched. Linear nanopolymers such as HPMA or polymalic acid carry functional groups distributed over the entire length of the polymer; branched polymers such as dendrimers usually carry them at the molecule surface. In micelles or other nanoparticles, oligomerization or aggregation would restrict accessibility and thus functionality of internally located groups.

Nanopolymers for personalized therapy

A large number of polymers are available, but only a few currently qualify for drug delivery in personalized therapy. Among the first nanopolymers successfully introduced in tumor treatment are HPMA conjugates that can deliver one or more different chemotherapeutics, notably doxorubicine, paclitaxel, geldanamycin and platinum derivatives [1,2]. The conjugates contain groups, which function in drug release, targeting and protection. HPMA-based nanopolymers have demonstrated efficacy in treatment of xenogeneic rodent models of human cancer and have achieved clinical trials. Their use in treatment of metastatic breast cancer is the subject of a recent review [3]. Another synthetic group of nanopolymers are

dendrimers. These are highly branched molecules with their star-like structure offering a versatility of functional groups at the polymer periphery to attach drugs and targeting molecules [3]. A principle concern with synthetic delivery platforms is incorporation of undesired material during polymerization and the elimination of the frequently nonbiodegradable carrier after treatment. These concerns are avoided by using biologically and enzymatically produced polymers, such as highly purified polysaccharides, polyamino acids and polymalic acid. Polysaccharides, such as dextran [4], and especially chitosan [5], have been developed into molecular nanocarriers to deliver drugs and nucleic acids, in particular, siRNA [6]. However, these nanopolymers remained primarily as the first-generation nanodrugs (carrying a prodrug and/or one additional functional group). However, after their hydrophobization through reactions with small molecules as well as with poly(lactic-*co*-glycolic acid) [7], polysaccharides can be assembled into nanoparticles (e.g., micelles, spheres and gels) capable of multifunctional drug delivery. Similarly, except when hydrophobized as above, polyamino acids, notably polyglutamic acid, have not evolved beyond the level of first-generation drug delivery nanopolymers. Nevertheless, polyglutamic acid–paclitaxel (CT2103) for treatment of $HER2^-$ metastatic breast tumors, went through Phase I–III clinical trials [8]. A poly(g,l-glutamic acid)–cisplatin conjugate effectively inhibited human breast tumor xenografts in nude mice [9]. High molecular weight poly-g-glutamic acid purified from natural sources has been found to be immunostimulating [10].

The multifunctionality of nanopolymers bearing various antisense oligonucleotides has been demonstrated by highly efficient treatment of glioblastoma [11], $HER2^+$ [12] and triple-negative [13] breast tumors *in vivo,* and with temozolomide treatment of brain cancer cells *in vitro* [14].

Personalized medicine & multi-targeting nanodrugs

Traditional chemotherapy is being gradually complemented or replaced with targeted molecular therapeutics since the discovery of molecular heterogeneity among tumors with identical histology [15]. Tumor gene profiling has shown genetic divergence in parts of the same solid tumor and its metastases in one patient [16]. High expression of epithelial-to-mesenchymal transition genes in breast tumors correlates with high enrichment of tumor-initiating cells and mammary stem cells [17]. Aspects of personalized therapy in breast cancer have already been considered in the light of targeted therapy using biomarkers [18]. Among modern drug formulations, nanopolymers are highly promising vehicles for multi-targeting and can provide molecular combination therapy and, thus, personalized therapy designed to treat individual tumors with specific marker expression profiles.

Nanobiopolymeric drugs crossing multiple biobarriers & inhibiting several molecular targets

Polymeric drugs with attached peptides or proteins binding to receptors on the endothelial cell have an advantage of being extravasated by transcytosis. This way, they cross the endothelial barrier and, after targeting specific proteins expressed on tumor cells, enter cancer cells via receptor-mediated endocytosis [11]. A recent example of a multi-targeted nanopolymer is polymalic acid loaded with anti-transferrin receptor (TfR) antibody

(ensuring transcytosis), Herceptin (binding to HER2 receptor and blocking its functional activity and signaling), and anti-*HER2* antisense oligonucleotides that block HER2 mRNA and HER2 receptor synthesis. This nanobiopolymer of the polycefin family achieved more than 90% growth inhibition of $HER2^+$ human breast cancer *in vivo* in a mouse model [12]. Another example is polymalic acid loaded with anti-TfR antibody (for transcytosis), 2C5 antibody (binding nucleosome-related antigen typically expressed by cancer cells for tumor cell targeting) and anti-EGFR antisense oligonucleotide to block the growth of triple-negative breast cancer [13]. This dual antibody targeting is particularly impressive for delivery. TfR-specific antibody mediates polymer permeation through endothelial barriers for breast [12,13] and brain cancers (brain–tumor barrier) by transcytosis through tumor vessels [11]. Extravasation by receptor-mediated transcytosis together with 'passive' extravasation generated by tumor-specific leakage of blood capillary and incomplete lymphatic drainage (enhanced permeability and retention effect) is followed by 2C5-mediated tumor cell-specific targeting [13].

A prerequisite of successful drug delivery by nanopolymers is drug release into the cytoplasm of recipient cells through endosomal membranes. This endosomal escape is especially important for highly negatively charged polymers such as HPMA, polymalic acid and polyglutamic acid, which cannot penetrate membranes without the aid of special accessories, such as cell-penetrating peptides [19]. However, many cell-penetrating peptides have toxic effects by acting on cell membranes. In cancer treatment, pH-responsive escape devices have been used, which are active only in late endosomes and lysosomes ($pH \approx 5$) or have been proven nontoxic at treatment conditions. Examples of escape devices for polymalic acid-based drug delivery are leucyl ethylester (pH-independent) or trileucine ($pH \approx 5$ f or activity) [20].

Conclusion

Nanopolymers with platforms, such as HPMA, dendrimers and polymalic acid, already available for multidrug delivery and multi-targeting of cancer markers together, can support safe and efficient drug delivery for personalized therapy of breast cancer. A polymalic acid nanopolymer platform, with proven biodegradability, absence of toxicity and immunogenicity, allows the design of regimens for systemic cancer treatment with multiple drug administration. These possibilities for a new generation of nanopolymeric drugs fit the idea of transforming cancer from a lethal to a chronic and potentially curable disease. Nanopolymer composites such as micelles, nanoshells, nanoparticles or liposomes with uncontrollable disassembly or reorganization after injection seem to have less potential for multiple systemic treatment in comparison with single nanopolymers that present pharmacological, immunological and therapeutic security to treat genetically unstable cancer cells.

Future perspective

Biodegradable and nondegradable nanopolymers have been developed, which are able to carry multiple drugs (in the form of prodrugs) and prevent tumor growth through inhibiting multiple cancer biomarkers. The polymers with chemical structure to attach several different

anti-cancer inhibitors will allow designing drugs for personal therapy at different stages of cancer progression or development for individual patient monitoring of his/her tumor genetic profile by delivery of complementary gene silencing molecules and/or chemotherapeutic drugs. In the simplest case, the tumor resistance to a delivered drug will be eliminated by codelivery of a second drug, either chemotherapeutic or gene targeting. If information is available about several cancer markers for a given tumor, these will be silenced simultaneously by corresponding inhibitory oligonucleotides or other drugs deliverable on each single platform molecule for a combination therapy effect.

With the molecular tumor characterization by analyzing fresh or archived tumor for validated or putative predictive biomarkers, and using techniques such as gene sequencing, gene expression microarrays, FISH or immunohistochemistry, the patient's tumor genetic/phenotypic profile can yield a number of biomarkers to be suppressed. In this respect, multifunctional targeted nanopolymers may be one of the best tools for future treatment.

Acknowledgments

This work was supported by grants from the NIH (R01 CA123495, R01 CA136841 and U01 CA151815 to JY Ljubimova), Winnick Family Foundation Clinical Scholar award (to JY Ljubimova), and Martz Breast Cancer Research Fund (to E Holler). The authors are shareholders of Arrogene Nanotechnology, Inc.

Biographies

Julia Y Ljubimova

Eggehard Holler

References

1. Lammers T. Improving the efficacy of combined modality anticancer therapy using HPMA copolymer-based nanomedicine formulations. Adv. Drug Deliv. Rev. 2010; 62:203–230. [PubMed: 19951732]
2. Kopecek J, Kopecková P. HPMA copolymers: origins, early developments, present, and future. Adv. Drug Deliv. Rev. 2010; 62:122–149. [PubMed: 19919846]
3. Lee JH, Nan A. Combination drug delivery approaches in metastatic breast cancer. J. Drug Deliv. 2012 (Epub ahead of print).

4. Varshosaz J. Dextran conjugates in drug delivery. Expert Opin. Drug Deliv. 2012; 9:509–523. [PubMed: 22432550]
5. Patel MP, Patel RR, Patel JK. Chitosan mediated targeted drug delivery system: a review. J. Pharm. Pharm. Sci. 2010; 13:536–557. [PubMed: 21486530]
6. Rudzinski WE, Aminabhavi TM. Chitosan as a carrier for targeted delivery of small interfering RNA. Int. J. Pharm. 2010; 399:1–11. [PubMed: 20732398]
7. Wang JJ, Zeng ZW, Xiao RZ, et al. Recent advances of chitosan nanoparticles as drug carriers. Int. J. Nanomedicine. 2011; 6:765–774. [PubMed: 21589644]
8. Li C, Wallace S. Polymer-drug conjugates: recent development in clinical oncology. Adv. Drug Deliv. Rev. 2008; 60:886–898. [PubMed: 18374448]
9. Yea H, Jina L, Hua R, et al. Poly(g,l-glutamic acid)–cisplatin conjugate effectively inhibits human breast tumor xenografted in nude mice. Biomaterials. 2006; 27:5958–5965. [PubMed: 16949149]
10. Poo H, Park C, Kwak M-S, et al. New biological functions and applications of high-molecular-mass poly-g-glutamic acid. Chem. Biodivers. 2010; 7:1555–1562. [PubMed: 20564573]
11. Ding H, Inoue S, Ljubimov AV, et al. Inhibition of brain tumor growth by intravenous poly(b-l-malic) acid nanobioconjugate with pH-dependent drug release. Proc. Natl Acad. Sci. USA. 2010; 107:18143–18148. [PubMed: 20921419]
12. Inoue S, Ding H, Portilla-Arias J, et al. Nanobioconjugate inhibition of HER2/neu signaling and synthesis provides efficient mouse breast cancer treatment. Cancer Res. 2011; 71:1454–1464. [PubMed: 21303974]
13. Inoue S, Patil R, Portilla-Arias J, et al. Novel nanobioconjugate inhibiting EGFR expression in triple negative breast cancer. PLoS ONE. 2012; 7(2):E3107.
14. Patil R, Portilla-Arias J, Ding H, et al. Temozolomide delivery to tumor cells by a multifunctional nano vehicle based on poly(b-l-malic acid). Pharm. Res. 2010; 27:2317–2329. [PubMed: 20387095]
15. Yap TA, Workman P. Exploiting the cancer genome: strategies for the discovery and clinical development of targeted molecular therapeutics. Annu. Rev. Pharmacol. Toxicol. 2012; 52:549–573. [PubMed: 22235862]
16. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N. Engl. J. Med. 2012; 366:883–892. [PubMed: 22397650]
17. Herschkowitz JI, Zhao W, Zhang M, et al. Comparative oncogenomics identifies breast tumors enriched in functional tumor initiating cells. Proc. Natl Acad. Sci. USA. 2012; 109:2778–2783. [PubMed: 21633010]
18. Stopeck AT, Brown-Glaberman U, Wong HY, et al. The role of targeted therapy and biomarkers in breast cancer treatment. Clin. Exp. Metastasis. 2012 (Epub ahead of print).
19. Koren E, Torchilin VP. Cell-penetrating peptides: breaking through to the other side. Trends Mol. Med. 2012; 18:385–393. [PubMed: 22682515]
20. Ding H, Portilla-Aries J, Patil R, Black KL, Ljubimova JY, Holler E. Polymalic acid peptide copolymers: design and optimization for endosomolytic drug delivery. Biomaterials. 2011; 32:5269–5278. [PubMed: 21514661]