

NANOTECHNOLOGY:
FUTURE APPLICATIONS OF BIOMARKERS

BY
HANNAH FARLEY
PASS WITH DISTINCTION

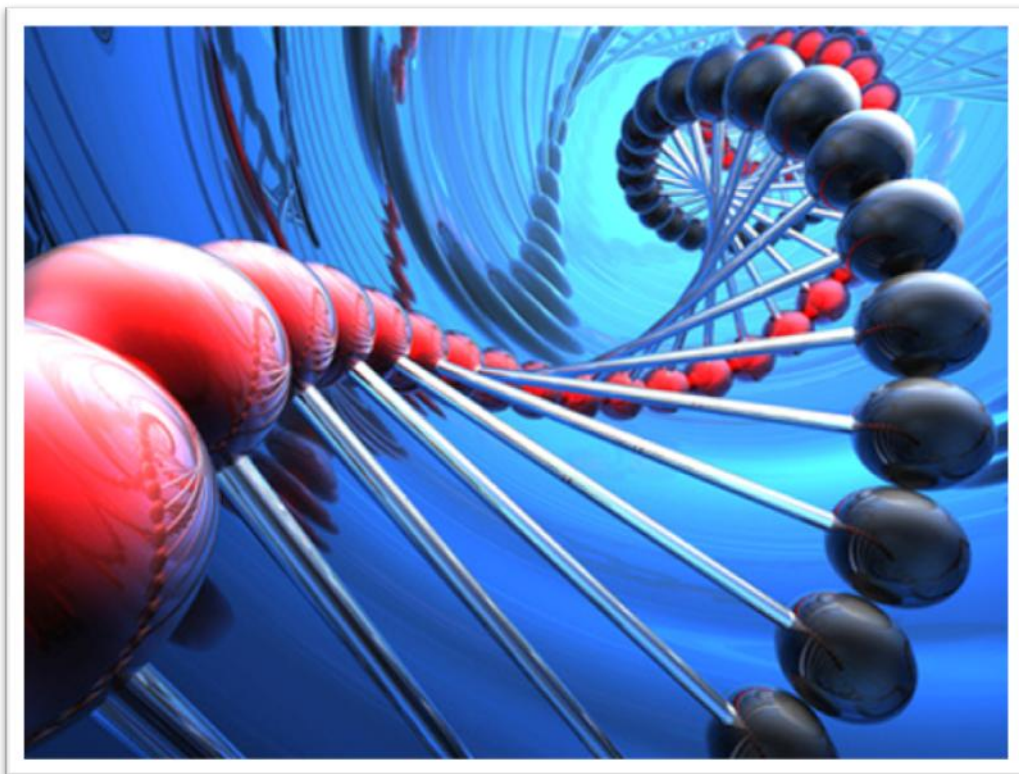


Fig. 1 Graphical representation of DNA – many biomarkers utilise our knowledge of proteomics to attach to specific cells

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT MEDLINK 2010

Abstract

In this paper, I will draw upon current medical research in the field of nanotechnology and important health care issues in the UK to propose future areas of research. These areas will focus on the possible applications of biomarkers, Qdots and related nanotechnology in destruction of atheroma and virally infected cells. I will also touch upon the ethical considerations that must be taken into account, and the potential of biomarkers for allowing us to understand more about diseases purely from a research point of view.

Introduction

Nanotechnology is a relatively novel area of scientific research in that it is only several decades old. Even in that short time, we have already seen huge advances in the fields of drug delivery and diagnosis. The crucial factor of nanotechnology that gives us so many opportunities – its unique selling point – is that for the first time, clinicians can work with diseases on a molecular level. We no longer need to affect the entire body when, for example, the tumour is only in the breast. We can prevent symptoms from occurring by detecting and defeating pathogens earlier, a complete change in the way medicine as a scientific discipline works.

As mentioned previously, one of the breakthroughs in medical nanotechnology came in the form of drug delivery. The development of new molecules such as the buckyball gives us the possibility of trapping free radicals, thought to be responsible for aging, whilst the advance of drug design as utilised by Dugan et al (2008) means that we could target potent and toxic medicines so that they only affected the areas or organs that we wanted them to. In the case of the so called buckyball, Dugan et al have adapted the fullerene molecule by adding carboxyl groups, and have shown that this adaption protects neurons from the effects of free radicals. Much of this work is down to our understanding of the cell membrane and proteomics, allowing us to create specific receptor sites on drug “packages”. Researchers are also trying to create a nanoparticle envelope that could withstand the digestive tract, allowing medicines currently only available in injection form, for example insulin, to be administered orally.

Another key breakthrough is the use of Qdots, or quantum dots, in diagnostic techniques. We can now attach a Qdot to a section of specifically coded protein. As Qdots emit photons when excited, this means that we can quite literally “see” diseased areas very easily, without the large doses of radiation used in other diagnostic methods. Again, our knowledge of proteomics is key to this. We have to know a specific receptor protein to attach the Qdot to so that we only end up “seeing” the targeted matter, for example a tumour. And even in older diagnostic techniques, nanotechnology can play a crucial role, with iron oxide nanoparticles covered in targeted peptides being used to improve the imaging of brain tumours gained from MRI scans.

Nanoparticles are also being used in an altogether more exciting matter in the treatment of cancers. As nanoparticles are incredibly small particles with a large surface area: volume ratio, they are generally more reactive and exhibit the properties of their bigger sibling compounds in greater ferocity. Nanoparticles can attach to cancerous growths due to their larger blood vessels can be stimulated to vibrate by X-ray or infrared waves, upon which they will cause the cell they are attached to die as shown by the work of Bhatia et al(2009). This provides a new alternative to current radiotherapy, with much less damage to the surrounding tissue.

Having covered the current research into medical applications of nanotechnology, I am now going to present the topic that the discussion will cover. It builds upon the work just discussed – a replacement for radiotherapy. I am going to combine this work with research on specific peptide receptors to demonstrate the fields into which I think nanotechnology should venture in the future; specifically, the destruction of diseased tissues in the body from inside, rather than out.

Discussion

First of all, I will cover the logic behind my choices for future nanotechnology research. Cancer is widely accepted as one of the biggest killers in the Western world, and another widely accepted fact is that its treatment is harrowing, and gives its patients a very poor quality of life. This is why I was so struck by the possibility of direct delivery of cancer drugs, and in particular by the current work that is ongoing with the use of nanoparticles in radiotherapy. As most chemotherapy drugs try to damage the DNA of the cancerous cells enough to prevent them from replicating, there is then an obvious problem if the DNA of the cancerous cell has mutated so much that it is not affected – it cannot commit apoptosis, and so we cannot kill it. Considering this, the use of targeted radiotherapy through nanoparticles gives brilliant prospects to future cancer patients, with a greater likelihood of survival and indeed a lack of recurrence.

How does this relate to my chosen research areas? Well, I'd like to take this research a step further. Another threat to health in the Western world, and indeed increasingly throughout the globe, is blood clots. Stroke and heart attack are generally fatal or disabling conditions that have a severe impact on not only the patient, but their friends, family, and the economy – as the population of our country ages, we will see more of these conditions, as their prevalence increases with age. Being able to regularly “clean up” our arteries and remove plaques before symptoms present or further complications develop would save many lives and greatly reduce the threat of conditions caused by blood clots. This treatment could happen annually or biannually, and although in the long term, convincing the nation to follow a healthier lifestyle would lead to more health benefits; this would be a cheap solution for clot caused diseases.

My supposed research avenue is that of taking the biomarker technology that we have previously seen used with Qdots – the peptide binding – and using this to bind to a different type of cell entirely, the foam cells that make up blood clots that cause strokes and heart attacks. This would serve as the scout of the “scout and assassin” technique of attacking cells as researched by Bhatia et al. We would then send in the “assassin” – a gold nanoparticle inside a polypeptide that would bind to the scout. When excited by infrared waves, the gold nanoparticles vibrate enough to destroy the cell they are attached to. Although this may seem like “far out” science, it is really only combining current research avenues of nanotechnology to show us what the future may well hold.

Previously, researchers such as Greenburg et al (2010) have demonstrated the utility of using biomarkers in the diagnosis of a range of diseases. Here, they used Qdots as the particle that their polypeptide chains were bound to in order to give quick diagnostic responses. We would use the same principle of a peptide receptor to attach uniquely to foam cells that accumulate under the lining of the artery, causing atherosclerosis. Already obvious problems are appearing. How do we find a specific receptor for foam cells, and how do we deliver the drug through the arterial wall? This is why we need nanotechnology.

Foam cells are essentially macrophages and T-cells that have reacted with oxidised low density lipoproteins (LDLs). The cells then cannot process the LDLs, which is why they accumulate under the arterial wall. If we can find a receptor for these cells, then we can solve this problem. My theory is to build a receptor unique to the oxidised low density lipoproteins. This process would be made simpler by the ability of drugs manufacturers to make models of drugs and their action in 3D – using drug design, yet another example of the action of nanotechnology. These programmes would allow us to more readily construct and test our scout and assassin models.

When we use infrared radiation as previously discussed to excite the nanoparticle attached to the receptor, the combined molecule will effectively explode. The violent shaking of the targeted protein would break bonds in the molecule, destroying the cell around it. This would not only break up plaques, but timed with an injection of a drug to “mop up” any LDLs left in the bloodstream, would remove the threat of clots for some time. I believe that the shattering of the foam cell would

contain the energy of the “explosion”, limiting the damage to surrounding tissues. I also think that, as with current radiotherapy technology, we now have the ability to fine tune the amount of radiation a patient receives. This would mean that we could supply enough radiation to just explode the foam cells, nothing more.

Although this technique would take more research to develop and then perfect, I feel that the possible outcomes are in effect worth it – and the end result would be achieved faster if we pool current research data and techniques. As this technique of “scout and assassin” radiotherapy treatment is already in trials to treat cancer cells, there are only a few unique concerns that remain to us – phase I and II trials for any application of this “explosion” therapy will quickly reveal if the damage to surrounding cells is simply too much for the body to take, as well as already reassuring us regarding the immediate toxicity of gold nanoparticles.

Key research avenues that would need to be explored to fully utilise this treatment would focus on the magnitude of the therapy. In patients with severe coronary heart disease, would we be able to start this treatment at all, or would the shock of so much therapy be too much? We could of course combine treatment with health checkups, as screening and vaccinations are currently, to ensure a slow and steady approach as opposed to a sprint starting when the patient has already suffered a clot caused disease. This could be integrated into previous government proposals for health MOTs, reducing the number of emergency scenarios that present in A and E wards, thus decreasing the financial strain of coronary heart disease on the NHS. Whilst pursuing this research, we would also need to answer the question of what exactly would remain in the body after treatment.

In coronary heart disease, the foam cells expand, accumulating more LDLs until they burst. The amount of LDLs then released into the blood stream means that more white blood cells are attracted to the area, more foam cells are formed and the problem is only exacerbated. We would need to ensure that the oxidised LDLs themselves were broken up, or else find another drug that could be injected at the same time to neutralise their threat. Otherwise, we would only be creating more accumulations of foam cells around the body, defeating the point of the therapy. However, as we have the specific questions that need to be answered already, I feel that this would simplify any future research dramatically.

As for the passage of the drug through the arterial wall, this poses more of a problem. Although the endothelial cells separating the lumen and the plaque are comparatively thin, how can we get our new drugs – both the scouts and assassins – across them? Well, the answer lies in “Drug Delivery into the Arterial Wall: A Time-Course Study with Use of a Lipophilic Dye” by Macke et al (1993) – as long as the injected drugs are non-polar, i.e. lipophilic, they are taken up by the arterial wall within 5 and 50 minutes of injection. This means that we must first inject the scout, wait an hour, inject the assassin, wait another hour or two to be safe and then induce vibration of gold nanoparticles using infrared radiation. As the “scout” polypeptides will only bind to oxidised LDLs in foam cells, when the attached “assassin” gold nanoparticles are activated, they will also destroy any bound polypeptide chains from the treatment that were not uptaken and are circulating in the blood stream. The idea is that these will then be filtered out by the liver, utilising the body’s usual metabolic pathways. Making our polypeptides lipophilic will be fairly easy to test due to drug imaging software.

Having already explained in detail what this treatment would entail, and the further research that would need to be undertaken for treatment of this sort, I will go on to give further possible applications of biomarkers in the targeted treatment of disease.

First of all, I feel that using biomarkers, we could tackle virally infected cells using the same principles as above. There are, however, bigger issues here. The question of whether the body could “take it” becomes ever more important, especially in the cases of diseases such as HIV/AIDS, where the function of the type of cell that would have to be targeted to beat infection is actually crucial to the body. The only way forward I can see here would be to find a protein that has changed on virally

infected cells, and build a receptor to it. Even then, we would still have the issue of mutating viral DNA to deal with. This avenue of research would take a long time, a lot of effort and money to pursue, but could offer hope to those affected by currently incurable viral conditions, again for example, HIV/AIDS.

Another possibility would be research into debilitating neurological conditions such as Parkinson's Disease (PD) and Alzheimer's dementia. Both diseases are caused by the build up of protein plaques or tangles, and especially in the case of PD, knowing more about the protein build up could prove useful in developing new treatments, or even one day a cure. Currently, we only know about the plaques and tangles from autopsies of patients or animals with the condition induced. This means that we know very little about the way the plaques are formed. By using fluorescing Qdots to attach to the proteins that make up these bodies, we could look at the way the disease progresses in patients by using regular brain scans to activate fluorescence.

Ethical issues with nanotechnology

I will now move on to discuss further the ethical implications of the development of nanotechnology, and the implications of the use of nanotechnology as already covered in this paper.

Primarily, the ethical issue here is one of beneficence and non-maleficence. Questions have been raised previously by the UK government about the safety of nanoparticles, prompting them to ask the Royal Society and the Royal Academy of Engineering to form a working group that has called for a more coherent approach. What if they get out of control? Will humanity cease to exist under a flood of gray goo created by miniscule robots, as famously hypothesised in *Engines of Creation*. The answer, as far as I can tell, is a resounding no, at least for the gray goo. The question does remain however, of whether doctors and researchers are acting in the best interests of their patients. Especially with diseases that are debilitating and costly, it can be tempting to only see the good that can come of a treatment. But can nanotechnology actually be actively harmful to a patient? Some scientists believe that nanoparticles could accumulate in the body and go on to cause cancer or other diseases of the genome as shown by Bhabra et al (2009). If so, we would then be guilty of focussing so much on the principle of beneficence that we have forgotten the principle of non-maleficence – it is all well and good to want to treat or cure a patient, but we would need to step back and remember that we would not want rash action that aids the patient now to hinder them or indeed their offspring later, as in the case of the thalidomide.

Then we come to other ethical arguments. As with stem cell research, do we as human beings have the right to interfere in nature to such an extent – to act as God and make our own building blocks to play with? Due to the current fad in nanotechnological research, that question becomes solely one of scholarly debate. So much research occurs in the field of nanotechnology that an ethical issue will not stop it. But indeed, if we are ignoring whether or not we have the right to behave in this way, are we willing to take responsibility for any problems we may cause? As the dominant conscious species on this planet, we have the responsibility to preserve it for future generations; to refuse to do so would defeat the point of our biological existence, namely to survive as a genome. What if we unwittingly unleash toxic nanoparticles - if the gold nanoparticles used as the “active ingredient” in the above research are filtered out of our bodies, only to pollute the surrounding environment and kill many organisms? Personally, I feel that although trials to repudiate this must take place, we already pollute our environment and our ability to change and solve problems has allowed us to survive this far. I find it hard to believe that pollution from nanoparticles alone would completely decimate humanity.

We must also discuss the principle of justice in relation to nanotechnology. The world is currently seeing a rapid growth in the prevalence of lifestyle and chronic illnesses, namely cancer, in previously developing countries that have now “boomed”, like India. The rapid increase in the

standard of living in these countries means that people can live much longer, and so are more likely to suffer from cancers and other illnesses that become more prevalent with age. At the moment, these patients are not offered treatments that are standard in nations that industrialised a century or more ago, for example the USA, UK and France. As a result, many patients with these diseases are condemned to death, even if their condition would be treatable had they lived elsewhere. The number of these cases is only likely to increase.

I feel that although this by no means is a reason to stop nanotechnological research, it does show that if we can, as a result of it, cure many types of cancer as an example, we have a moral duty to deliver this medicine worldwide. It is difficult to picture people dying from diseases that we can treat so easily. Even though it happens all the time, and certainly happened long before the advent of nanotechnology, that does not nullify the ethical question that is raised by any scientific advance. Surely all scientific research aims to seek some benefit. With medical research, this benefit is generally for the good of humankind. Can we truly say that nanotechnology is a worthwhile investment of precious scientific resources (facilities, researchers and indeed money) when it will not reach its full potential unless we can tackle the wider issue of global medical coverage?

Conclusion

To conclude, I feel that nanotechnology is definitely one of the most promising scientific avenues open to research in our present day and age. I am also optimistic for its future – the rapid advances it has seen combined with the “Star Trek” science that it can make possible makes it a well advertised and well funded area of science. The possibilities do seem very great. I am almost certain that as research into all aspects of nanotechnology continues, we will see some doors close – but we will also see others open, and the fact remains that nanotechnology could give us the ability to tackle diseases that twenty years ago were a death sentence.

However, I would urge caution to researchers in this field. No one of us would want nanotechnology to be tarnished by rushed testing and ecological or health and safety disasters as a result, something that I believe could well happen due to the excitement and huge investment this area of science has seen, notwithstanding the massive profit margins almost certainly to come.

I feel that the applications of biomarkers discussed in this paper could well come to fruition; indeed, the technologies that these applications are based on are well under way regarding testing and large scale trials. I also hope that scientists will use nanotechnology to continue to solve existing problems, rather than create new ones – applications in the use of nanotechnology as a research or diagnostics tool could be much more profitable and effective than saying straight out that we will have a twenty year research project aimed at using nanotechnology to eliminate viruses.

Finally, I believe that we can make these benefits accessible to all, and indeed, it is our moral duty to do so. I strongly feel that if ever the time comes when we can use nanotechnology to eradicate some of the most powerful killers on the planet, we should ensure that we do so everywhere and not just in countries fortunate enough to be able to pay for the treatment directly.

References

1. Ali S. S., Dugan L. L., Hardt J. I. (2008) SOD Activity of carboxyfullerenes predicts their neuroprotective efficacy: a structure-activity study. In the journal of Nanomedicine: Nanotechnology, Biology and Medicine, 4 (4), 283-294
2. Beckman, K. B., Bruce N. A. (1998) The Free Radical Theory of Aging Matures. Physiological Review, 78 547-581
3. Bhabra G. et al (2009) Nanoparticles can cause DNA damage across a cellular barrier. In the journal Nature Nanotechnology, 4, 876-883
4. Bhatia S. N. et al (2009) SERS-Coded Gold Nanorods as a Multifunctional Platform for Densely Multiplexed Near-Infrared Imaging and Photothermal Heating. In the journal of Advanced Materials, 21 (31), 3175-3180
5. Bhatia S. N. et al (2009) Computationally Guided Photothermal Tumor Therapy Using Long-Circulating Gold Nanorod Antennas. In the journal of Cancer Research, 69 (9), 3892 - 3900
6. Chen X., Lee S., Liu G., Swierczewska M. (2010) Functional nanoparticles for molecular imaging guided gene delivery. In the journal of Nano Today, 5, 524-539
7. Chenglong L., Huameng L. (2010) Multiple ligand simultaneous docking: Orchestrated dancing of ligands in binding sites of protein. In the Journal of Computational Chemistry, 31 (10), 2014-2022
8. Consigny P.M., Miller K.T. (1994) Drug delivery into the arterial wall: a time-course study with use of a lipophilic dye. In the Journal of vascular and interventional radiology, 5 (5), 731-737
9. Forlorni G. et al (2002) Protein misfolding in Alzheimer's and Parkinson's disease: genetics and molecular mechanisms. In the journal of Neurobiology of Aging, 23 (5), 957 - 976
10. Gee A. D. (2003) Neuropharmacology and drug development: Imaging in clinical neuroscience. In the British Medical Bulletin, 65 (1), 169-177
11. Kruth H. S. (2001) Macrophage foam cells and atherosclerosis. In the journal Frontier of Biosciences, 6 (1), 429-455
12. Manoharan N., Samal S. S., Vishwakarma V. (2010) Safety and Risk associated with Nanoparticles: a review. In the Journal of Minerals & Materials Characterisation & Engineering, 9 (5), 455-459
13. Varghese C. Cancer Control and Prevention in India
14. Drexler K. E. (1986) Engines of Creation, New York, Anchor Books
15. Faergeman O. (2003) Coronary Artery Disease, Amsterdam, Elsevier Science
16. Gold nanoparticles in cancer treatment <http://web.mit.edu/newsoffice/2009/gold-cancer-0504.html>
17. Cancer information <http://www.cancerresearchuk.org/>
18. Cardiovascular disease and its prevalence in LEDCs <http://www.who.int/mediacentre/factsheets/fs317/en/index.html>
19. The action of chemotherapy drugs <http://www.patient.co.uk/health/Chemotherapy.htm>
20. Kain K., Greenberg M. and Qdot diagnostics <http://www.ontariogenomics.ca/research/project/34>
21. Atherosclerosis <http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001224/>
22. Computer programmes aiding drug design <http://www.netsci.org/Science/Compchem/feature09.html>
23. Radiation therapy and its use in treating cancer <http://www.cancer.gov/cancertopics/factsheet/Therapy/radiation>
24. Government public health proposals <http://news.bbc.co.uk/1/hi/health/4015733.stm>
25. Our incomplete understanding of Parkinson's Disease, p15 http://www.parkinsons.org.uk/pdf/Progress_Winter2010-11.pdf
26. The Royal Society and Royal Academy of Engineers working group <http://www.nanotec.org.uk/>
27. Thalidomide survivors <http://thalidomidesurvivors.org/>
28. http://www.strokecenter.org/education/ais_pathogenesis/03_role_monocytes.htm
29. http://www.itqb.unl.pt/Research/Technology/Biomolecular_Diagnostics/Activities/?link=5
30. <http://www.genengnews.com/analysis-and-insight/biomarkers-poised-for-breakout-moment/77899322/>