diagnostic kits, based on a biotechnological approach. Combining the techniques derived from electronics, that allow smaller samples to be analysed and the development of miniaturised sensors and nanobiodevices (such as lab-on-a-chip) it becomes possible to simultaneously measure a large number of parameters from one sample (high-throughputscreening) so providing the physician an extended set of data helpful for diagnosis and prognosis.
Nanotechnology can be critical in improving biochip technology offering novel ways to design and create biochip architecture, surface coatings and the production of building blocks for small devices or nanoscale optics to allow the study of individual or collective properties of luminescent biological responses at molecular level. By using fragments of DNA or antibodies as sensing agents, for example, it is possible to better understand the molecular basis of diseases and identify the molecular targets for therapy. The advantages of the introduction of nanotechnology are lower costs, improved performance and reliability and extreme miniaturisation.
Nanoanalytical tools for in vitro diagnosis (imaging) include also a wide spectrum of ancillary techniques such as scanning probe microscopy or imaging mass spectrometry, which can be used in molecular pathology or for reading highly integrated biochips. Non invasive, in vivo diagnosis at the molecular level based on targeted imaging, is another fundamental aspect of nanomedicine. It can allow the early detection of an illness, monitor its progress and control the response to treatment. Molecular imaging refers to the visual representation, characterization, and quantification of biological processes at the cellular and sub-cellular level within intact living organisms.
Targeted molecular imaging is essential for pinpointing the localisation of diseases as well as the distribution and accumulation of the drugs. By combining imaging technology and drug delivery systems there is the possibility to realise theranostic devices.
Quite a large number of nanoparticles are already available for targeted imaging diagnosis. Quantum dots and dye-doped silica nanoparticles are used as tracers in optical imaging with living tissues and can be particularly usefulfor toxicological investigation. Superparamagnetic iron oxide nanoparticles and dendrimers are used as MRSI imaging agent. Liposomes, emulsions, microbubbles, linear polymers, are more and more used for advanced targeted imaging diagnostic.
In the area of therapy, nanotechnology offers a great opportunity for the development of new, more effective, drug delivery systems for biologically active drugs. Improved drug delivery is, in fact, an important goal of modern medicine and much attention is focused on targeted delivery, improved safety and faster development. The availability of nanotechnological carriers that specifically deliver the drug where it is needed could prevent unwanted side effects. Targeted drugs that specifically kill cancer cells without the side effects of conventional chemotherapy should improve efficacy and reduce dosage. Nanosized drug carriers have already been developed and include liposomes, inorganic nanoparticles (ex. gold, silicates, magnetic nanoparticles), polymers coniugates, polymer-based gene delivery systems, nano-crystals (ex. Nano-sized milled drugs). The number is expected to grow as the spectrum of material adapted drug delivery is very ample and includes nanocapsules, nanoemulsions, micelles, nanotubes, nanogels, nanofibres. In the medium term targeted drug delivery is expected to evolve to systems that involve multiple action such as the combination of diagnostic imaging and therapy.
Another field in which nanotechnology can play an important role is regenerative medicine. Tissue engineering, based on cell culture techniques and bio resorbable polymers can be used to repair/replace damaged body tissues, promises to be the new frontier of this branch of medicine. Considering the evolution of European population tissue engineering could provide effective remedies for pathologies associated with ageing.
In conclusion, the parallel use of nanotechnology and biotechnology in nanomedicine can bring forward unprecedented tools for health care which can greatly improve medical practice.

On the web:

http://www.airi.it

Novel cell based technologies for drug discovery

Marcella Chiari, C.N.R.

Drug discovery programs of novel pathogenesis associated targets require the setting up of different steps for the identification, validation, and optimization of the compounds that can enter the clinical phase.
The overall process is costly and time consuming, also considering that, when the lead candidate enters the clinical phase, often it does not fulfill the requirements of a successful therapeutic product.
Moreover the majority of new drugs approved each year, in particular for cancer disease, are directed against known targets whereas only few lead compounds are against novel targets.
This reflects the criticism associated with the drug discovery approaches and suggests that a better improvement of the drug discovery pipeline could be achieved by using screening methods that identify the key molecular targets, rather than hit the pathways that are associated with the disease development.
Technologies like gene targeting, in which the gene can be selectively inactivated or knocked-down by RNA interference approaches (RNAi) can be really powerful in this context; such screenings are set to interrogate the genome either in a genome-wide scale of in a gene-family scale for specific disease associated phenotypes.
The RNAi approach is an effective and powerful method for the silencing of specific proteins: the read-out of this process will be evident at the level of cell phenotype, especially when the silenced genes participate in essential cellular processes.
Different new, cell-based, approaches for gene targeting by RNAi for drug discovery have been proposed[1]. Arrayed screens are performed either in a multi-well format o in a microarray based format, while pooled library screens target many genes as a "pool" in solution. Among them, arrayed screenings on microarrays, although very challenging, offer potential advantages in terms of reproducibility and costs reduction for cells and reagents. These approaches rely on technological challenges

Fig.1: RNAi and cell microarray based assays in the Drug Discovery pipeline, for the identification, validation and optimization of novel drug candidates.

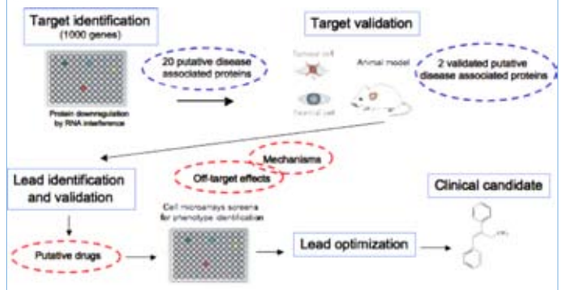
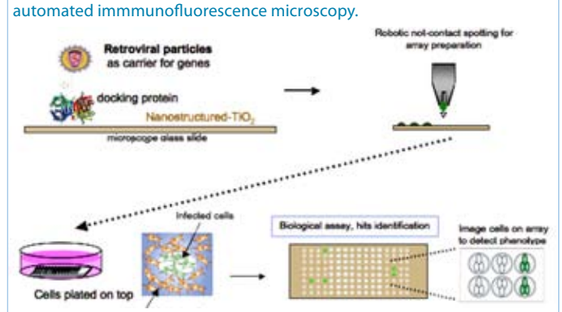


Fig.2: Retroviral cell microarrays: schematic representation of chip preparation and assay development: cell phenotypes are then analyzed by automated immmunofluorescence microscopy.



where different disciplines like material science, molecular and cellular biology must be integrated to develop functional and efficient assays.
Cell microarrays based approaches can be considered an "high tech" tool **(Fig.1)** that can be used in the drug discovery pipeline at different levels: potentially cell microarrays could identify novel targets that will require classical validation on "in vitro" and "in vivo" assays; furthermore, after compound identification, cell based microarrays could be used to identify mechanisms and off-target effects of the putative drug, before the compound enters the clinical phase: this would results in an increase of specificity of the compound and in a more detailed characterization of the action mechanism, al least in the "in vitro" context, before the evaluation in the clinics.
So far, few examples of phenotype screenings, using cell microarrays technology, have been proposed; the reason of the slow diffusion of this approach is mainly due to the technological complexity associated with the setting up of the chip and the limitation in cellular targets suitable for the assay.
The successful development of a cell based microarray device relies on different features: first of all, the chip must be biocompatible and suitable for long term cell adhesion; second, the biomolecules for genetic transduction (plasmid or viruses) must be correctly immobilized on the surface without loss of transduction efficiency; third, the technology should benefit from advanced surface patterning to restrict biomolecule's functionalization and cell adhesion on precise areas. Ultimately, the robustness of the technology will depend on the efficiency of genetic and localized transduction as well as on process automation.
Cell based microarrays will be ready to enter the phase of wide application in complex screenings programs only upon the achievements of the above mentioned requirements.
Moreover, miniaturization and automation are the key features to adequately respond to the post-genomic needs; cell based microarrays are among the most suitable technologies to approach such needs.

References:
Iorns E, Lord CJ, Turner N, Ashworth A. Utilizing RNA interference to enhance cancer drug discovery. Nat Rev Drug Discov. 2007 Jul;6(7):556-68
Carbone R, Giorgetti L, Zanardi A, Marangi I, Chierici E, Bongiorno G, Fiorentini F, Faretta M, Piseri P, Pelicci PG, Milani P. Retroviral microarray-based platform on nanostructured TiO2 for functional genomics and drug discovery. Biomaterials. 2007 Apr;28(13):2244-53
Barborini E, Piseri P, Milani P. A pulsed microplasma source of high intensity supersonic carbon cluster beams J Phys D: Appl Phys, 32, 1999
Carbone R, Marangi I, Zanardi A, Giorgetti L, Chierici E, Berlanda G, Podestà A, Fiorentini F, Bongiorno G, Piseri P, Pelicci PG, Milani P. Biocompatibility of cluster-assembled nanostructured TiO2 with primary and cancer cells Biomaterials, 27, (17) 2006

On the web:

http://www.tethis-lab.com

http://www.genextra.com

Novel cell microarray- based assay

Recently Tethis has proposed a novel cell microarray based assay[2] (fig.2): the technology relies on the powerful capability of retroviruses to genetically modify target cells to knock-down families of genes for evaluating specific phenotypes; retroviruses are arrayed on a novel chip coated of nanostructured TiO2 (TIDOXc) (3, 4) and the functional assay can be performed for different kind of disease associated phenotypes, for short and long term assays.
The technology has been validated either by overexpressing a GFP reporter gene or by downregulating p53 through RNAi. In addition the extent of p53 downregulation and its biological relevance in the context of a DNA-damage assay was addressed on human primary cells. As expected, primary cells displayed an altered cell growth phenotype, as a consequence of p53 down-regulation. These data suggest that Tethis' technological platform is an efficient tool for phenotype screenings in primary and cancer cells for different applications, i.e. when long-term expression of the transgene is required and antibiotic selection of infected cell clusters is necessary to obtain homogeneous populations of genetically modified cells. In addition, the viral production protocol is suitable for automation with robotic liquid handlers in 96-well plates, allowing to be scaled up for the employment of arrayed libraries of retroviruses.
The unique properties of the novel TIDOXc chip are related to the capability of immobilizing the retroviruses in arrays, allowing efficient gene transduction in different primary and cancer cells, and to its biocompatibility that permits cell culture of cells even for long term assays. Due to these features Thetis' technology overcomes previous limitations in cell microarrays based approaches: furthermore it benefits from the features of miniaturization and automation providing a cost effective approach for RNAi based screenings either for the identification of novel drug targets, the evaluation of mechanisms and off-targets effects of putative candidate compounds. (Patent pending: "Materials and supports for in vitro bioassays" WO2007/009994)