Abstract

The shared mission of the biological, medical, surgical, and public health disciplines pursued under the auspices of the Department of Biomedicine and Prevention is to study new prevention methods and procedures through biomedical research, and how to effectively implement these in Italy’s national health service. The Department also investigates new risk factors, biomarkers, models, and techniques for both the prevention and treatment of common and rare diseases.

To that end, basic research conducted by the Department has produced numerous in vitro and in vivo experimental models for the characterization of processes that regulate embryonic development. These studies have enabled the delineation of several molecular processes aimed at controlling the processes involved in DNA methylation, cell cycle progression, and response to drug treatments. Models for in vitro screening of new molecules with potential pharmacological activity have also been produced.

With regards to infectious diseases, the molecular mechanisms of HIV infection have been investigated and, with a focus on evaluating predictive factors of drug response, the developmental effects on breastfed babies of mothers undergoing treatment for HIV have been studied.

Studies into degenerative diseases have mainly focused on neurological and degenerative disorders (atherosclerosis and diabetes). In particular, experimental models have been developed that have enabled the characterization of certain molecules (frataxin) and genes (SMN) involved in Friedreich's ataxia and spinal muscular atrophy respectively.

Genetic studies conducted have helped to establish the role played by certain genes in human diseases such as diabetes mellitus type 2 and spinal muscular atrophy.

Numerous anatomo-clinical studies, evaluating the expression of specific biomarkers correlated with plaque vulnerability, have shown that organ damage from acute cerebrovascular syndromes is correlated to thrombosis of a carotid plaque, rather than to the degree of vessel stenosis.

In the field of oncology, more recent studies have been aimed at identifying potential biomarkers of the onset and/or progression of the disease. In particular, certain proteins involved in different stages of tumor progression (CLIC1 in glioblastoma; FMRP in breast cancer; and clusterin in colorectal cancer) have been characterized. In addition, some microRNAs have been identified as tumor markers (miR-221/222; and miR-128).

Numerous experimental studies have enabled innovative therapies to be made available in the diagnostic, medical, and surgical fields. Of particular note, a recent study has shown that acute promyelocytic leukemia (APL) can be beaten without chemotherapy.

Research into new imaging techniques combined with computational modeling and massive parallel computing has allowed non-invasive diagnostic methods to be developed for the diagnosis and characterization of the microstructural alterations underlying many neurodegenerative diseases, as well as for the analysis of liver, breast, and prostate disorders, and for quantifying the risk associated with repeated head trauma. Furthermore, the application of radiofrequency models has enabled the development of protocols for the percutaneous ablation of breast and liver tumors.

Research conducted in the field of regenerative medicine and surgery has enabled the development of cutting-edge methods for using synthetic biomaterials, stem cells obtained from adipose tissue and other tissue, and growth factors contained in platelets. In addition, mini-invasive surgery techniques have also been developed both for thoracic and vascular surgery with a view to reducing operative risk in patients.
Special attention has also been focused on interactions with environment. In this regard, interactions with environment and humans of particulate matter produced by new technologies (nanotechnologies) have been studied. Research has also been conducted into the biocompatibility of synthetic materials to facilitate the in vivo regeneration of damaged tissue.

In the field of service organization and delivery, a strand of research worthy of mention has focused on prescription and treatment adherence, health education, and training of medical and health personnel, involving analysis of healthcare approaches most apt to bring about a more rational use of scarce resources, and hence create potential savings for hospitals and publicly-run hospital facilities.
RESEARCH OUTPUT

From the establishment of the Department to date (2011-2013), the research activity conducted has enabled many scientific works to be produced involving numerous international collaborations. The research works produced during the period 2011-2013 are set out in Attachment 1 to this report (containing an itemized list detailing IF distributions, citations, relevant field of research, type of work produced, affiliations, and contemporary h-index). In summary, based on Scopus queries performed on December 10, 2013, the following citation metrics were retrieved for the Department:

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REPORT ON RESEARCH ACTIVITY (2011-13)

All our Department’s research activities have the shared goal of finding specific responses to the questions posed by clinical practice in the field of infectious, degenerative, and neoplastic diseases. To achieve this, there are a number of strands of research in place that can be grouped into the following five main thematic areas:

- Regenerative medicine and surgery;
- Modeling for:
  - Infectious diseases (HIV, TB, malaria, and others)
  - Non-infectious diseases
  - Neurological disorders and imaging
  - Service organization and delivery
  - Interactions with environment;
- Biomarkers and predictive factors of disease;
- Genetics and epigenetics applied to aging, lifestyle, and environment; and
- Rare diseases: innovation in their prevention and treatment

1. Regenerative medicine and surgery

Regenerative medicine and surgery together constitute the most recent applications of medical science aimed at restoring the function and integrity of damaged parenchyma and tissue. This relatively “minimally invasive” approach uses patients’ own cells and tissue, enabling them to make very rapid postoperative progress. This reduces physical and mental stress and accelerates the healing process. The research conducted in this field has made cutting-edge methods available for the use of synthetic biomaterials, stem cells obtained from adipose tissue and other tissue, and growth factors contained in platelets.

2. Modeling

2.1. Infectious diseases

Studies on HIV infections in high prevalence areas such as sub-Saharan Africa have focused on predictive factors of therapeutic response, cost-effective models for universal screening, and the developmental effects on breastfed babies of mothers undergoing treatment for HIV. In particular, the effects of triple antiretroviral drug therapies during pregnancy and breastfeeding were evaluated weighing determinants such as viral load, CD4, BMI, co-infections (especially TB), defensins in milk, anemia, viral resistance to drugs, and genetic predisposition to adverse drug events.

Through the DREAM (Drug Resource Enhancement against AIDS and Malnutrition) program, large cohorts of adults have been studied for factors associated with mortality (especially premature death), loss to follow-up, and treatment failure. These studies have enabled the development of a predictive mathematical model based on semi-Markov chains aimed at evaluating the goal of universal access to testing and treatment set by the WHO.

Studies aimed at investigating the molecular mechanisms of HIV infection have shown that ADAR enzymes (which, in vertebrates, bind double-stranded RNA molecules and catalyze hydrolytic deamination reactions that convert adenosine into inosine, helping to finely regulate gene expression) stimulate different stages of HIV-1 replication, thereby functioning as proviral factors. In addition, researchers from our Department have identified certain cellular microRNAs which show substantial changes in their gene expression as a consequence of HIV-1 infection.

Other studies in the field of infectious diseases have enabled the development of innovative enteric virus detection
models to control infections from sewage sludge and effluents of certain wastewater treatment plants.

2.2. Non-infectious diseases

The morphological and molecular characterization of embryonic germ-cell development is of fundamental importance to the understanding of many pathogenetic mechanisms of neoplastic and degenerative diseases. The research conducted in this field has produced many experimental models concerning the development of germ cells and the extracellular matrix, epigenetic modifications, and response to carcinogenic agents. In particular, studies on the organization of the cumulus oophorus extracellular matrix have enabled the identification of two proteins necessary for the organization of hyaluronic acid in the cumulus oophorus extracellular matrix that surrounds the ovulated oocyte. It has been demonstrated that the deletion of these genes in mice alters the formation of this matrix and determines sterility in the female in a knock-out mouse model for proteins involved in the organization and integrity of the cumulus oophorus extracellular matrix.

Several researchers focused their interest on the systems that modulate embryo implantation. Using mice as an experimental model, the endocannabinoid system in blastocysts and uterine tissue was identified, pinpointing this system as an important modulator of embryo implantation processes. The endocannabinoid system (ECS) – which includes the cannabinoid receptors CB1 and CB2, endogenous cannabinoids, and the enzymes that synthesize and degrade endocannabinoids – has also been identified and partially characterized in embryonic stem cells. The studies carried out have demonstrated that the CB2 receptor plays a role in promoting progression and entry into meiosis of spermatogonia.

Germ cell development has also been studied, characterizing the epigenetic mechanisms involved in the development of mammalian germ cells. These results have contributed to the characterization of epigenetic modifications such as DNA demethylation and histone demethylation/methylation which play a primary role in primordial germ cell development in mammals. These processes are responsible for the reprogramming of the genome that characterizes the development of the germ line from the time of formation of PGCs until the maturation of gametes. The studies in this area have contributed to the analysis of the dynamics of DNA demethylation and the characterization of the enzymes that control these processes in pregonadal and gonadal PGCs.

In terms of characterizing the molecular mechanisms involved in neoplastic disorders, the recent identification of microRNAs has allowed the identification of a complex network of non-coding regulatory genes that perform various functions in oncological diseases. In particular, the role played by a pair of microRNAs expressed in tandem (miR-221 and miR-222) as “magnifiers” in glioblastoma and prostate cancer has been studied. It has been shown that miR-221/222 increase the proliferative potential of in vitro models of human prostate cancer by inhibiting translation of the negative regulator of the cell cycle p27Kip1. The ectopic inhibition of miR-221/222 is capable of significantly retarding the growth of human prostatic tumor xenografts in murine animal models. In addition, the functional characterization of Myc-modulated microRNAs in cancer stem cells of glioblastoma has made it possible to demonstrate that certain microRNAs play a key role in cell proliferation and tumorigenesis of glioblastoma multiforme (GBM). Recent findings have shown that microRNAs are important components of the Myc oncogene pathway. Experiments conducted in tumor stem cells isolated from human GBM have highlighted that modulation of Myc function entails a profound change in the expression pattern and function of miRNAs involved in the onset and growth of GBM.

Various studies have been designed to improve understanding of the molecular mechanisms underlying cellular response to drug therapies. In particular, studies have been conducted into testicular germ cell tumors (TGCTs) that are very sensitive to cisplatin-based therapy due to a defective homologous recombination (HR).

In terms of experimentation aimed at discerning new pharmacologically-active molecules, a model has been developed, in collaboration with a pharmaceutical manufacturer (Sigma-Tau), for the screening of small molecules that mimic specific peptides situated in key regions of proteins involved in innate immunity – such as, for instance, the MyD88 protein – so as to interfere with their function and block “signaling” of “Toll-like receptors” (TLRs). It is interesting to note that this receptor has an altered function in various human diseases (for example, Waldenström’s macroglobulinemia and multiple sclerosis).
2.3 Neurological disorders and imaging

Various neurological disorders still lack specific treatment, including Friedreich’s ataxia (FA), a genetic neurodegenerative disease. A research group attached to our Department has observed that interferon gamma increases the levels of frataxin in cells derived from patients with FA. In preclinical studies on FA mouse models, it has also been shown that treatment with interferon gamma increases the levels of frataxin in the neurons of spinal ganglia, slowing their degeneration and improving the motor-coordination performance of affected mice. Certain mechanisms of frataxin degradation have also been clarified, with molecules developed of potential pharmacological use capable of interfering with such degradation, as a strategy for raising frataxin levels. Some of these compounds have shown effectiveness in determining an increase in cellular frataxin levels in cells derived from patients with FA. Another research group in our Department has focused its attention on studying the genetic basis of neurological diseases. In particular, it is recalled that molecular studies have been conducted to characterize the mechanisms that regulate the loss of function of the SMN1 gene responsible for motor neuron degeneration in spinal muscular atrophy. These studies have enabled the identification of a key regulator of “SMN2 exon skipping” in fibroblasts of patients with spinal muscular atrophy. Non-linear signal processing techniques were also used for the quantification of aberrations in neurovascular coupling in nocturnal frontal lobe epilepsy and temporal lobe epilepsy, demonstrating a parallelism between the precritical phase and physiological arousal.

In recent years, the vast quantity of new knowledge produced in the fields of medicine and biology has significantly altered the possibility of using diagnostic imaging techniques as necessary and indispensable aids in the diagnosis, prognosis, and monitoring of disease, and in carrying out both diagnostic and therapeutic interventional procedures. Research conducted in the field of imaging has enabled non-invasive diagnostic methods to be developed for the diagnosis and characterization of the microstructural alterations underlying multiple sclerosis, glaucoma, hereditary spastic paraplegia, Alzheimer’s disease, mild cognitive disorder, leukoaraiosis, Kennedy’s disease, and Marcus Gunn phenomenon, as well as for the assessment of liver fibrosis, the detection and characterization of neoplastic focal liver lesions, metabolic imaging of prostate cancer, and quantifying the risk associated with repeated head trauma through MR spectroscopy. The application of radiofrequency models has enabled the development of protocols for percutaneous ablation of breast and liver tumors. The integration of advanced multimodal neuroimaging protocols (based on resting-state fMRI, diffusional kurtosis imaging, and SPECT/PET), used in synergy with “machine-learning” and massive parallel computing techniques, has enabled predictive characterization of individual patients with regards to vascular dementia (vascular MCI) and Parkinson’s disease, demonstrating also that with this condition the residual levels of endogenous dopaminergic transmission can serve as a predictor of cognitive response to treatment.

Imaging techniques such as 3D ultrasound and 3D power Doppler enable placental volume and vascularization in the first trimester of gestation to be assessed. Studies conducted in this area have shown that changes in these parameters as pregnancy progresses exhibit differences in pregnancies at risk of hypertensive disease, intrauterine growth retardation, and type 1 diabetes.

2.4 Service organization and delivery

In the field of service organization and delivery, a strand of research worthy of mention has focused on prescription and treatment adherence, health education, and training of medical and health personnel within the DREAM (Drug Resource Enhancement against AIDS and Malnutrition) program, active since 2002 in the fight against AIDS in sub-Saharan Africa (with over 150 thousand HIV+ patients assisted). In assessing adherence, the main indicators in use were studied and evaluated. The research done in the area of health education focused on interventions of potential benefit to persons with a low level of education/schooling. In addition, programs have been developed for distance learning, access to second-opinion consultations, and telemedicine services in collaboration with the Azienda Ospedaliera San Giovanni-Addolorata in Rome. In conjunction with the non-profit organization “Medicina Solidale” in Rome (since 2010) and the “Genti di Pace” medical clinic in Rome (since 1985), monitoring and evaluation is carried out on access to Italy’s national health service by persons belonging to vulnerable groups (of Italians, foreigners, and Roma). Ad-hoc tools have also been developed and used for the purposes of research relating to health education (HIV, hypertension, diabetes, and so on), and to primary (HIV) and secondary (TB) prevention. Within the ambit of the “Innovation in health approaches” project, analysis has also been undertaken of healthcare
approaches most apt to bring about a more rational use of scarce resources, and hence create potential savings for hospitals and publicly-run hospital facilities.

2.5. Interactions with environment

It is well-known that fine particulate matter has harmful effects on human health (such as pneumoconiosis). A strand of research has been developed within our Department dealing with the interaction with environment and humans of particulate matter produced by new technologies (nanotechnologies). This line of research is funded by the European Commission (under the Seventh Framework Programme) as part of the MARINA (Managing the risk of nanomaterials) project and covers potential negative effects, particularly as regards reproduction and embryonic development, as well as possible beneficial effects, such as use in the biomedical field. In respect of the latter area, studies have been developed on the biocompatibility of synthetic materials and their use as tridimensional scaffolds for the maintenance and differentiation of embryonic and adult stem cells, with the aim of improving in vitro culture conditions of these cells by providing them with a synthetic tridimensional matrix on which to grow, and, in future, to utilize such bioreabsorbable scaffolds to facilitate in vivo regeneration of damaged tissue.

With a view to reducing the environmental impact of disinfecting chemical agents, analyses have also been performed on antimicrobial activity in vitro in order to evaluate the disinfectant efficacy of an ozonated oil-based product for disinfecting the hands of healthcare workers.

3. Biomarkers and predictive factors of disease

The term “biomarker” is used to refer to “any biological indication” correlatable to the presence of a given disease and/or predictive of the evolution or response to the treatment thereof.

3.1. Genetic disorders

The “Biomarkers regulation and qualification at the EMA (European Medicines Agency)” research unit within our Department is involved in studying, promulgating, and consulting with partners on EMA directives and guidelines for the qualification and regulation of new (particularly genomic) prognostic and pharmacogenetic biomarkers identified and validated within the context of the project and associated interactions with the EMA (FP7, “Health” theme, Dec 2009-Dec 2012) Grant Agreement “Identifying and validating preclinical biomarkers for diagnostics and therapeutics of neuromuscular disorders”). A study conducted on genetic variability in a population of patients with IBD was aimed at identifying susceptibility and/or protective polymorphisms for the disease, as well as identifying susceptibility/protective polymorphisms common to CD and UC and other inflammatory diseases, such as psoriasis, systemic lupus erythematosus, and rheumatoid arthritis, in addition to variants associated with particular phenotypes and complications. The study of genetic variability in relation to response to drugs and research into pharmacogenetic biomarkers pursued two main lines of inquiry: 1) genetic variability associated with the variability of response in patients treated with the anticoagulant warfarin; and, 2) genetic variability associated with the development of side effects (hepatotoxicity and Stevens-Johnson syndrome) in HIV patients treated with the antiretroviral drug nevirapine.

3.2. Neoplastic disorders

In the field of oncology, one of the most studied topics was tumor progression. In particular, research in collaboration with the VIB/KU Leuven, in Belgium, has helped explain the way in which the fragile X syndrome protein (FMRP) contributes to breast cancer progression. FMRP acts as a molecular “switch” that is capable of controlling the levels of other proteins involved in different stages of the progression of breast cancer, such as the spread of cancer cells in the bloodstream and the invasion of other organs to form metastases.

In terms of tumorigenesis, study of the tumorigenic potential of cancer stem cells derived from human glioblastomas has enabled characterization of the role of CLIC1 (chloride intracellular channel 1). A marked up-regulation of CLIC1 was recently described in the type of glioblastomas characterized by the worst prognosis. In collaboration with a
research group at the University of Rome "La Sapienza", our research team characterized the role of a specific microRNA, miR-128, in in vitro models of human neuroblastoma. It was shown that miR-128 is able to inhibit the expression of two proteins, DCX and reelin, which promote migration and invasiveness of neuroblastoma cells. The study of microRNAs in neuroblastoma was subsequently expanded, resulting in the identification of microRNAs useful as prognostic markers of this disease.

Generating much interest recently is research into early disease markers of both diagnostic and predictive value. In particular, anatomo-clinical studies in patients with colorectal cancer have helped reveal an “up-regulation” of sClusterin, a pleiotropic protein with a broad distribution and range of functions. An oligoclonal antibody patented by researchers in our Department has made it possible to detect the increase in excretion levels of this protein in the blood and feces of patients with colorectal carcinoma.

3.3 Degenerative disorders

Atherosclerosis, a degenerative disease of the blood vessels, is responsible for cardio- and cerebrovascular diseases that are the leading cause of death in industrialized countries. Many anatomo-clinical studies have amply demonstrated that acute cerebrovascular syndromes are pathogenetically correlated to rupture and thrombosis of a vulnerable carotid plaque, rather than to its degree of stenosis. Carotid plaque at high risk of rupture, generally termed “vulnerable” as distinct from stable plaque, is characterized by an extensive lipid/necrotic core covered by a thin fibrous cap which is the site of an “active” chronic inflammation mainly involving T lymphocytes and macrophages that are stimulated, through the release of inflammatory cytokines, to activate lytic enzymes that determine the thinning and rupture of the cap. The collaborative work of several groups attached to our Department has led to the identification “in situ”, in human carotid plaques, of the expression of specific markers correlated to plaque vulnerability, which characterize various developmental stages of the disease. The identification and characterization of markers correlated specifically and sensitively to the instability of carotid plaque is the main target for identifying subgroups of patients at high risk of acute ischemic events to be identified.

3.4 Functional disorders

A study on the role of anti-Müllerian hormone (AMH) in the diagnosis of polycystic ovarian syndrome (PCOS) conducted in around 60 infertile women with PCOS, with suspected PCOS, and without PCOS (control group), set out to ascertain the threshold value of AMH that enables a diagnosis to be made of polycystic ovaries (PCO) in women with PCOS. The correlation between blood levels of AMH and other conventional indicators of ovarian reserve, both hormonal (FSH, LH, 17 beta-estradiol, inhibin B) and ultrasound-measured (ovarian volume, and number of antral follicles of 2-9 mm in diameter), was assessed. Also evaluated was the AMH threshold value (representing the best compromise between sensitivity and specificity) that enables a diagnosis of PCOS to be made. This study showed that the AMH test has a diagnostic potential for the diagnosis of PCOS in the absence of hyperandrogenism or anovulation and/or where a follicle count cannot be obtained.

4 Genetics and epigenetics applied to aging, lifestyle, and environment

The transcription of the genome is a highly regulated process, in which transcription factors, proteins that bind to specific regulatory sequences on DNA, play a crucial role in activating or repressing the activation of individual genes and, lastly, the sequence of events that leads to the synthesis of proteins essential for cellular functions. Numerous studies conducted by researchers attached to our Department have helped clarify the role played by certain genes in human diseases, such as diabetes mellitus type 2 and spinal muscular atrophy. Among the many genes identified, one our Department’s research groups focused its attention on the LOX-1 gene: high expression of LOX-1 in the placenta suggests that this receptor plays a crucial role in placental function. It has further been hypothesized that LOX-1 is involved in the process of trophoblast invasion in the early stages of pregnancy and in accelerated apoptosis in preeclampsia. In addition, studies in murine embryos have revealed the existence of splicing isoforms of the murine homologue gene Olr1, specifically expressed during embryogenesis. Lastly, the interaction of LOX-1 with the NF-kB signaling pathway and particularly with various cytokines, such as IL-6 and TNF-alpha, makes it a candidate...
gene for a key role in the processes that underlie polyabortive pathogenesis. An analysis was also conducted on DNA breakpoints in genes involved in leukemias secondary to cytotoxic therapies. In particular, the model of acute leukemias developing after treatment of multiple sclerosis with mitoxantrone was studied. In addition to the genomic characterization of these patients, possible genetic predisposition was investigated through study of polymorphisms of genes involved in DNA repair (such as BRCA2).

5. Rare diseases: innovation in their prevention and treatment

Given that rare diseases and innovative treatment have considerable public health impacts, research in this area is high-profile. A group of researchers in our Department has identified a new syndrome called normal-weight obese (NWO) syndrome, which predominantly affects young women, and in which subjects, alongside weight and anthropometric measures within the normal range, present a reduced muscle mass associated with an increase in fat mass (30%), as well as having high blood concentrations of inflammatory cytokines. In addition, numerous studies have enabled innovative therapies to be made available in the medical and surgical fields. Of particular note, a recent study has shown that acute promyelocytic leukemia (APL) can be beaten without chemotherapy. In a GIMEMA-AIL study, a combination of retinoic acid and arsenic trioxide – hence, free of chemotherapy – was compared with the conventional approach of combining retinoic acid with chemotherapy. The results across more than 160 patients showed 2-year survival rates of 98% for patients treated with arsenic, compared with 91% for patients who had received chemotherapy. This therefore marks the first time that success has been demonstrated for a therapeutic strategy of treating acute leukemia based solely on targeted therapies.

5.1. Mini-invasive surgical treatments

In surgery, general anesthesia with single-lung ventilation can have many adverse effects, especially in older and/or co-morbid patients. Non-linear signal analysis techniques have accordingly been used to assess and predict the effects of general anesthesia on baroreflex sensitivity, and the occurrence of hemodynamic instability during transient events such as clamping and declamping the abdominal aorta, as well as to predict response to fluid infusion (“fluid resuscitation”). In order to reduce operative risk in patients with increased surgical risk, an ultra-mini-invasive approach has been developed, called “awake” thoracic surgery, which employs video-assisted thoracoscopic access carried out under local or epidural anesthesia with the patient conscious and breathing spontaneously. A new miniature surgical instrument has also been developed and patented recently which can be released within the thorax and can then be duly mobilized from outside the thoracic cavity without recourse to further surgical access. This type of technology could enable even complex video-assisted pulmonary surgical procedures to be performed through a single small incision.

The diseases that could benefit greatly from a minimally invasive approach include aneurysmal disorders. In addition to being useful for monitoring aneurysms, the technique can be employed for the treatment of complications arising from acute occlusion after endovascular therapy for infrarenal AAA. The use of Doppler ultrasound enables monitoring of the functionality of renal arteries and renal parenchyma. The test is able to integrate information obtained from other instrumental methods and has the advantage of being repeatable. Remaining on the subject of vascular disease, it should be recalled that DVT is the most formidable complication of prolonged postoperative clonostatism. Doppler ultrasound is the test of choice for the diagnosis and monitoring of patients with DVT. Laser treatment of the greater saphenous vein is the new frontier in mini-invasive treatment of venous disease. Research in this area has focused on: 1) deep system thrombosis, with evaluation of preventive measures and possible complications; and 2) insufficiency of the superficial venous system and an evaluation of laser treatment compared with surgery.