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Identification of new Predictive Osseointegration Bio-Markers of the prosthetic implant in patients with OsteoArthritis"

Study Code:	**Bio.Pre.I.OA**
Sponsor:	IRCCS - Istituto Ortopedico Rizzoli Via di Barbiano 1/10 40136 Bologna Italy
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Study methodology:	In vitro biological study with human derived matererial
Type:	No profit
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Background and study rational

Age-related degenerative disorders, such as osteoarthritis, are among the diseases with the greatest socio-economic impact; moreover, the increase in life expectancy of the population has led to an increase in the number of people affected by this disease in the last decades [Pelletier et al., 2001]. Osteoarthritis (OA) is a chronic joint degenerative disease characterized by degeneration of hyaline articular cartilage, synovial inflammation and changes in the subchondral bone. There are two forms of OA, primary and secondary; the latter occurs at a later age, tending after 60 years, and is due to the natural process of aging of the joint and / or to events of a traumatic nature. Several other etiopathogenetic factors are involved in the development of this degenerative process including genetic factors, obesity and chronic inflammatory status, and the complex pathogenesis is not yet fully determined. The joints most affected are those of the upper and especially lower limbs and the spine and the symptoms are algia, joint stiffness, muscle tension and instability, which induce the patient to immobility in an attempt to avoid pain; this symptomatology often aggravates the quality of life of elderly patients to produce a depressive state that further complicates the general clinical picture [Pelletier et al 2001; Hunziker, 2001]. The current pharmacological and physiatric treatments are not resolutive of OA and in fact, the prosthetic intervention turns out to be the only solution for the patient. In recent decades, the technology has developed more and more performing joint prostheses, inspired by different mechanical and biological principles, in order to better reproduce joint physiology and improve the efficiency of the implant. Currently the materials used have a long life span, and 90 to 95% of the operated patients have a prosthetic implant survival 10 to 15 years after surgery. However, implants can also fail for several reasons: dislocation, aseptic loosening, peri-prosthetic infections aggravated by sometimes predisposing factors of the patient, such as diabetes, which determine the need to perform a revision surgery [Pelletier et al., 2001]. In spite of this, chondrocyte has a key role in the degradation of hyaline cartilage by: i) an imbalance between its own anabolic and catabolic activity; and ii) to the reduction of the chemical-physical properties, other cellular components are more involved in the subsequent possible failure of the integration of the prosthetic implant. Modifications of the joint microenvironment, the deficiency of the regenerative response of the osteoblasts, the inappropriate activation of the chemochemical network and the activation of the synoviocytes in the inflammatory sense (activation of the pathways of NF-KB, HIF1, BMP, TGF-β, and IGF) they are all conditions and factors that can alter the osseointegrative process of a joint prosthesis [Pelletier et al 2001; Hunziker, 2001; Asahara H. et al., 2016]. The aim of the present study is to identify and evaluate the possible role of miRNAs and long non-coding RNA (lncRNA), modulated in OA, as new biomarkers useful to predict the efficiency of the osseointegration process of the prosthetic implant [Xu et al. 2016; Zhang et al. 2017; Bernard NJ. et al., 2014].

Objectives of the study

- Primary Objective

  Identification and characterization of miRNAs and LncRNA, from synoviocytes, osteoblasts and chondrocytes isolated from tissue samples obtained from endo or surgery artro-prosthesis, and related signaling involved in synoviocytes and osteoblasts cross-talkduring the osteointegration process through suitable *in vitro* co-culture models [Craig I.Jet al., 2016; Lin Y.et al., 2010].

- Secondary Objectives:

  Identification of other biomarkers useful for predicting the efficiency of osseointegration of the implant, through bioinformatics and molecular analysis of miRNAs, lncRNA and proteins secreted or produced by synoviocytes and osteoblasts;

  Validation of data obtained on miRNAs and lncRNAs through "gain and loss of" experiments function "in primary cultures and co-cultures.

Study design

The study involves the isolation and cellular, molecular and proteomic characterization of synoviocytes, osteoblasts and chondrocytes from ~~"waste"~~ tissue samples obtained from patients undergoing surgery of endo or arthroplasty for osteoarthritis (Experimental Group) or interventions for other causes such as for example periarticular fracture requiring the implantation of a prosthesis (Control Group) at the Rizzoli-Sicilia Department (Bagheria-Palermo).

Deeper analysis of miRNAs and lncRNAs expressed by the synoviocytes, osteoblasts and chondrocytes will be performed in order to understand their possible involvement in the signaling of HIF1-α, β-catenin, NF-Kb and other pathways of osteogenic differentiation, through suitable experimental *in vitro* models of co-cultures. In particular, we will focus on the cross-talk between synoviocytes and osteoblasts, cell populations involved in the post-surgical articular microenvironment and on which the osteointegration process of the joint prosthesis depends.

Population

Inclusion Criteria:

1) Male and Female patients > 40 years old hospitalized for surgery of endo- or arthroplasty for OA (Group OA) or for surgery for joint traumatic diseases (eg femoral neck fractures) requiring the implantation of a prosthesis (Control Group);

2) Interval of at least three months from any previous infiltrative treatment of any kind;

3) Patients able to provide written informed consent to the study.

Exclusion Criteria

1) Severe cognitive defects or psychiatric disorders;

2) Tumor pathologies or concurrent antineoplastic therapies;

3) Autoimmune diseases (rheumatoid arthritis);

4) Surgery that involves the joint

Material and Methods

The isolated cells (synoviocytes, osteoblasts and chondrocytes) will be kept in culture under specific conditions in order to allow the isolation of proteins, mRNAs, miRNAs and lncRNAs. Cellular characterizations will be performed using FACS (Partec CyFlow Space), while the molecular characterizations will be obtained with qRT-PCR (Light-Cycler 2.0-Roche). Protein / proteomic analysis will be performed using ELISA (commercial kit), Western blot (ChemiDoc XRS + System, BIORAD) and TRIPLE TOF5600 (AB Sciex). The miRNAs and lncRNAs, identified by molecular biology techniques (qRT-PCR, LINE GENE 9640), the mRNAs and the identified proteins will be analyzed through the use of specific software, such as: TargetScanPrediction, Diana Software, MiRBase and Partec FloMax®. The miRNAs and lncRNAs that will be particularly regulated / modulated following the pathology and the reduced activation of the pathways involved in osteoblastic differentiation, will be investigated, evaluating their involvement in the regulation of HIF1-α, β-catenin, NF-Kb signaling and other possible pathways of osteogenic differentiation. Through in vitro co-culture systems (osteoblasts and synoviocytes isolated from tissues), we will evaluate the role of previously identified miRNAs and lncRNAs, in cross-talk between osteoblasts and synoviocytes isolated from patients with OA. Their role will be validated through "gain and loss of function" experiments in primary cultures and co-cultures.

Statistics

Multivariate statistical analysis of the results will be performed using R software [R Development Core Team (2008) software. A: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.] After evaluating the normal distribution homogeneity of the variance of data the appropriate parametric statistical tests will be applied (ANOVA or GLM and Student t test) or in case of non-normality and heteroskedasticity similar non-parametric tests (eg Kruskal-Wallis and Mann-Whitney U test). The proteomics data will be analyzed by principal components analysis using MarkerViewTM 1.2.1 (SCIEX) software. The molecular interaction network between the modulated proteins that are found to be significant will be analyzed with the STRING software v10 (Search Tool for the Retrieval of Interacting Genes / Proteins; http://string-db.org/). The sample size was calculated using the G*Power software v.3.1.9.2 (University of Kiel, Germany), considering

a significance level $\alpha = 0.05$ and a power $1-\beta = 0.80$. For the calculation of the effect size we considered the result of variation of *ALPL* gene expression in human mesenchymal stem cells cultured in hypoxic environment (similar to the intra-articular environment) between 3 (FOI: 1.53 ± 0.34) and 7 (FOI: 2.53 ± 0.29) days and presenting an increased expression of miR-675-5p [Costa V. et al, 2017]. The effect size value d=2.28 was transformed into f=1.14, because the sample size was calculated considering a two-way ANOVA model (patient: healthy / osteoarthritis, experimental culture time: 2 short times). The maximum number of patients to be enrolled is n=10 of which n=5 osteoarthritic and n=5 healthy.

Enrollment Procedures

Patients considered eligible will be enrolled in the study, after providing a written informed consent.

Data Collection

Clinical data will be retrieved by patient's source document

A protocol-specific CRF reporting the results of the analyses will be provided

A CRF is required and should be completed for each included subject.

Ethics

The clinical trial protocol and its documents will be sent before initiating the study to the competent Authorities and Ethics Committees of each participating country for its approval.

The responsible investigator will ensure that this study is conducted in agreement with either the most updated Declaration of Helsinki and all the international and local laws that apply to clinical trials and to patient protection.

The protocol has been written, and the study will be conducted according to the principles of the ICH Harmonized Tripartite Guideline for Good Clinical Practice

(ref: http://www.emea.eu.int/pdfs/human/ich/013595en.pdf).

Informed Consent

All patients will be informed, by the investigator, of the aims of the study, the possible risks and benefits that will derive from the study participation.

The Investigator must clearly inform that the patient is free to refuse participation in the study and that can withdraw consent at any time and for any reason.

They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

The informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

The Investigator must also sign the Informed Consent form, and will keep the original at the site and a copy of the original must be handed to the patient.

The competent ethics committee for each Institution participating to the study must validate local informed consent documents before the study can be opened. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the study whenever he/she wants. This will not prejudice the patient's subsequent care.

General Principles for Human Biological Material (HBM) collection

Human biological material (HBM) collection involves the collection and storage of biological material, residual biological material or derivatives in compliance with ethical and technical requirements.

Biological material (tissue samples) will be centralized and stored at l'Istituto Ortopedico Rizzoli - Dipartimento Rizzoli-Sicilia, Bagheria (Palermo).

From here, the biological material will be used and stored according with the sample characteristic and applicable regulation.

The molecular and protein materials obtained from the isolated cells will be stored inside sterile containers (falcon, tubes, eppendorf) in the freezer at the temperature of -80 ° C at the Istituto Ortopedico Rizzoli - Dipartimento Rizzoli Sicilia.

The "Piattaforma Tecnologica per l'Ingegneria Tissutale, Teranostica ed Oncologia" laboratory will perform the research as stated in the protocol "Material and Methods" section

The following principles apply to storage of HBM:

- The Istituto Ortopedico Rizzoli will have a designated person responsible for collection and will act as a communication point
- The collected HBM should be documented, i.e. the amount remaining and its location. act as a communication point
- The storage and use of biological material will take place in accordance with the standards of good laboratory practice (GLP) and applicable legislation.

Confidentiality

In order to ensure confidentiality of clinical trial data as disposed the national and European applicable regulation, data will be only accessible for the trial Sponsor and its designees, for monitoring/auditing procedures, the Investigator and collaborators, the Ethics Committee of each corresponding site and the Health Authority.

Investigator and the Institution will allow access to data and source documentation for monitoring, auditing, Ethic Committee revision and inspections of Health Authority, but maintaining at all times subject personal data confidentiality as specified in the applicable regulation.

The Investigator must guarantee that patient anonymity is kept at all times and their identity must be protected from unauthorized persons and institutions.

All patients included in the study will be identified with a numeric code, so that no identifiable personal data will be collected.

The Investigator must have and conserve a patients' inclusion registry where it figures the personal data of the patient: name, surname, address and corresponding identification code into the study, this register will be kept on the Investigator File.

Publication policy and data ownership

The principal investigator of the study is responsible for the final report, of the publication publish all the data collected as described in the protocol and will ensure that the data are reported responsibly and consistently.

In particular, the publication of data deriving from the present study will take place independently of the results obtained.

The transmission or dissemination of data, through scientific publications and / or presentation at conferences, congresses and seminars, will take place exclusively following the purely statistical processing of the same, or in an absolutely anonymous form

All the study data are owned by the sponsor.

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