A BIOBANK SUPPORTING RARE DISEASE RESEARCH IN DERMATOPATHOLOGY. OUR EXPERIENCE IN ESTABLISHING A BIOBANK

BIOBANCA-SUPORT ÎN CERCETAREA AFECȚIUNILOR RARE DERMATOLOGICE. EXPERIENȚA NOASTRĂ ÎN ÎNFIINȚAREA UNEI BIOBĂNCI

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Abstract

Biobanks of human patient sample tissues and blood fractions are increasingly recognized as major assets in disease research. We aim to identify DNA copy number and gene expression aberrations typical of different cutaneous pathologies. Another goal is the identification of circulating biomarkers both as prognostic, therapy-responsive and/or therapy-monitoring factors and as disease classifiers and subclassifiers. We established a complex biobank, the first as such in Romania, based on fresh frozen material and formalin fixed parafine embedded specimen, backed up by an exhaustive database with focus on cases of cutaneous lymphoma and inflammatory diseases, but also basal cell carcinomas, squamous cell carcinomas, melanomas. At present, the biobank contains 320 patients peripheral blood ,tissue samples and extracted DNA specimens , with full authorization of the donors for use in research activities and approval by ethic committees and authorities. An important feature of our genomic data analysis is the integration of molecular data generated during our studies to results deposited in genomic data repositories (www.progenetix.net). We expect a high level of impact of our research for the development of diagnostic tools and identification of candidate molecules for targeted therapies.

Keywords:

biobank, research, samples, dermatopathology

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Rezumat

Cuvinte-cheie:

biobancă,
studiu, probe,
dermatopatologie

Biobăncile care prezervă probe proaspete de țesut şi sânge au devenit în ultimii ani din ce în ce mai recunoscute ca având un rol major în progresul cercetărilor medicale. Scopul proiectului nostru este de a identifica aberațiile care pot apărea în replicarea ADN şi expresia genică şi sunt sau pot fi implicate în apariția sau declanşarea unor diferite patologii cutanate. Alt obiectiv foarte important al acestui studiu este acela de a identifica biomarkeri celulari, tisulari şi circulanți, care ar putea avea un rol important în prognosticul, răspunsul la tratament şi monitorizarea evoluției pe parcursul tratamentului, dar ar putea ajuta şi la clasificarea sau subclasificarea mai precisă a unor afecțiuni. În cadrul acestui proiect de cercetare a fost înființată prima biobancă din România în care se păstrează probe proaspete de țesut cutanat conservate la minus 80 de grade Celsius, probe de sânge fracționat, specimene fixate în formol şi incluse la parafină, material genetic (ADN) extras, împreună cu o bază de date sub forma unui registru cu informații clinice despre pacienții incluşi în studiu. Colecția de probe de țesut cutanat se axează în principal pe tipuri rare de limfoame cutanate şi afecțiuni inflamatorii cutanate – dermatoze inflamatorii –, dar include şi alte neoplazii cutanate, precum melanoame şi carcinoame bazocelulare. În prezent, biobanca conține probe de sânge periferic, probe de țesut cutanat şi extract genomic de ADN de la 320 pacienți, obținute cu consimțământul scris al donorilor pentru a fi folosite în scop de cercetare, respectând toate normele etice impuse. Datele obținute în urma acestui studiu vor completa bazele de date internaționale prin intermediul platformei Progenetix (www.progenetix.net). Sperăm ca rezultatele cercetărilor noastre să aibă un impact major în dezvoltarea unor noi metode de diagnostic şi în identificarea unor noi molecule candidate pentru terapii țintite.

Introduction

Biobanks of patients' tissue specimen, corresponding blood samples and associated clinical data are increasingly recognized as major assets in clinical diagnostics and biomedical research. Here, we present the first biobank of dermatologic samples in Romania, established in the frame of a recently funded Swiss-Romanian research program. We present and underline aspects regarding the founding of a biobank based on our experience [(1)].

Quality of materials and operations

The biobank currently includes fresh frozen material, extracted genomic DNA, fractionated blood samples from 170 patients, as well as formalin fixed paraffin embedded specimen from 150 patients, with a supporting database of clinical information. The sample collection is focussed on rare types of cutaneous lymphoma and inflammatory skin diseases, but extends to other cutaneous neoplasia (basal cell and squamous cell carcinomas, melanomas) [(2)].

The fast growing collection contains full donor authorization and ethics committee approval for use in research activities. Patient consent is fundamental to the ethical guidelines for obtaining and storing samples. Our project aims to support external participants both through archiving submissions of material, and through enabling the use of the material and anonymized supporting information in research studies [(3)].

With regard to sample processing, our focus is directed on the use of standardized protocols covering all steps from sample extraction, preparation and fractionation to appropriate long term storage. The team involved consists of both senior and young researchers with multidisciplinary scientific backgrounds (physicians, biologists and biochemists). Sample collection and preparation follow previously established protocols [(4)]. We aim at collecting and processing participant samples as quickly as possible after extraction. Sample collection is performed in the clinical routine setting and the corresponding blood samples are processed in parallel and within a few hours by local

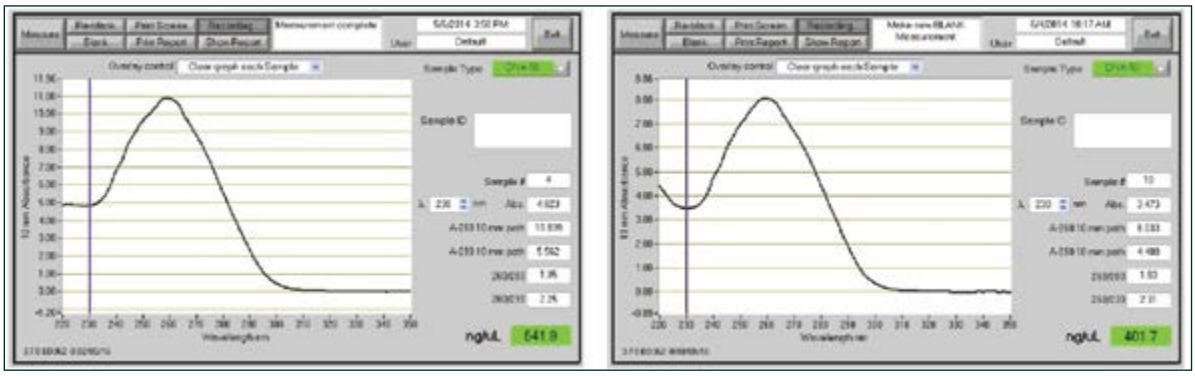

Figure 1. Representative DNA peak isolated from our FFPE samples (left) and fresh frozen samples (right)

laboratories. In surgically obtained tumor samples, two pathologists independently confirm a presence of at least 80% malignant cells by means of H&E staining prior to storage. For the tissue samples, time between collection and cryopreservation at -80° C is kept at a minimum to avoid degradation of labile specimen or components (e.g. RNA) [5].

Transparency (outreach activities and donor relations)

We believe that biobanks need to engage patients starting with well structured informed consent. This issue is central in our organization, and maintaining a strong link between the donor's consent and the use of his or her data is a legal and ethical obligation in the collection, storage and use of biospecimens and data for research purposes. All the samples in our biobank were collected with full donor authorization based on adequate information and mutual agreement for the proposed research and the modalities of individual participation [6].

The collection of biological material for diagnoses and research is of high and further increasing importance. Patient material for pathological, cell-biological, and molecular-biological investigations is essential [7]. For dermatology this concerns in the first-line skin as well as blood. This is related to the fact that several dermatological diseases seem to be systemical diseases and not entirely restricted to the skin.

Besides insuring a high quality diagnostic service, our intention is to also present our results in scientific papers published in prestigious MEDLINE and/or ISI indexed journals, communications in national and international symposia, conferences and congresses. We currently organize workshops to present and discuss our results with Romanian and foreign researchers and health care providers to obtain an extensive dissemination of our findings [8].

Usage (sample turnover)

One of our most outstanding features is that our biobank focusses in particular on rare skin diseases. In line with current scientific knowledge we have sampled, processed and are currently molecularly analyzing seldom inflammatory dermatoses assumed to progress to cutaneous lymphomas[9]. In an accompanying research study performed in collaboration with the University of Zürich, we specifically aim to:

- identify DNA aberrations and gene expression changes typical for different, previously understudied cutaneous pathologies
- investigate the tissue and circulating biomarkers (circulating immune cells, circulating cancer stem cells, immune molecules, chemokines, angiogenic and growth factors) related to the various aspects of skin diseass (clinical and histopathologic appearance, prognosis and treatment)
- transpose genetic data in reliable tissue & circulating biomarkers;
- establish an integrated guide to good practice for diagnosis/prognosis/therapeutic approaches in cutaneous pathologies.

To date, genomic DNA was extracted from 87 FFPE specimens using QIAamp DNA FFPE Tissue Kit and 75 fresh frozen materials using PureLink Genomic DNA mini kit. The elution buffer contains 10 mMTris-HCl and 0.1 mM EDTA. The absolute yield of extracted DNA was assessed using a NanoDrop ND-1000 (Figure 1). The spectrophotometric analysis resulted in an average 1.8 for A260/280, indicating that the extracted DNA from the biobank samples showed a high quality and was free from protein contamination.

To further confirm sampling quality and exclude DNA fragmentation, for sensitive microarray analytical processes, extracted DNA of fresh frozen materials is further and routinely validated by tight bands on 1% agarose gel electrophoresis. The sizes of well purified DNA fragments can be detected between 20 and 30 kb as confirmed in Figure 2.

Extracting DNA from FFPE tissues is generally regarded as a challenge. Based on available literature data we expect to obtain fragmented DNA, most fragments appear to be less than 400 - 600 bp in length. Predesigned TaqMan SNP genotyping products rs3892097 and rs1057910 (Life Technologies) are used to evaluate the FFPE DNA integrity. Real time PCR was performed in an ABI 7900 (Life Technologies) in 7 yl total volume with

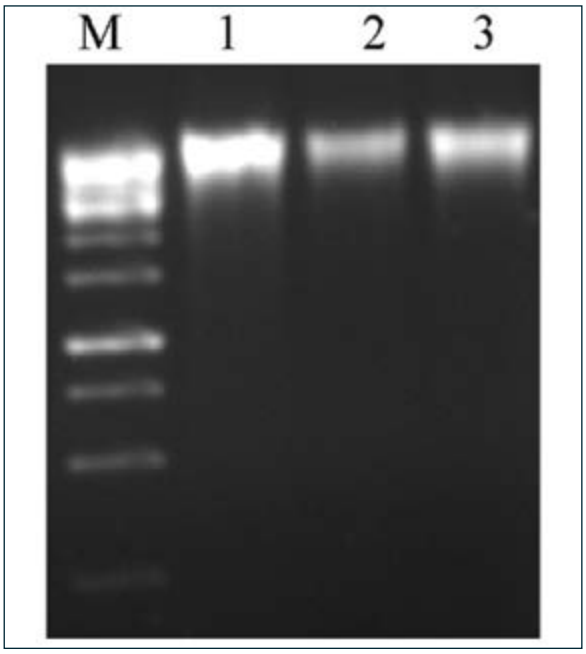

Figure 2. Electrophoretic analysis of total genomic DNA on 1% agarose gel (DNA from fresh frozen samples in line 1-3, molecular weight ladder in line M)

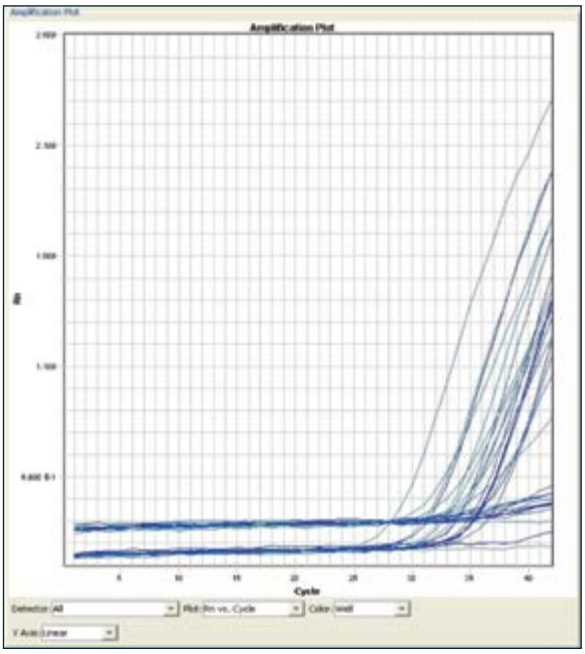

Figure 3. Representative Real Time PCR amplification curves for quality control of DNA extracted from FFPE samples stored in our biobank

1 ng of DNA/reaction. The mean Ct value was 34 (Figure 3), suggesting an excellent quality also for DNA isolated from the FFPE samples and thus supporting our strict storage conditions [10].

Connectivity (integration into Biobank networks and trans-institutional infrastructure)

All the people involved in the research processes benefit from collaboration with highly respected experts of impressive academic level and are exposed to a highly stimulating environment. The research teams are able to complete laborious and difficult tasks and the accumulated experience is the fundament for future projects and collaborations.

The integration with external datasets using the Progenetixplatform as well as the sharing with the research community through our web portal atcnhl.progenetix.org are important features intended for the molecular screening data generated in our studies. The Group at the University of Zurich is one of the leaders in the field of large scale meta-analyses of oncogenomic copy number data and maintains the largest collection of annotated CNA data in cancer (www.progenetix.net). Recently, the group has launched a reference resource for oncogenomic array data (www.arraymap.org). A continuous integration of original study data with related information is an element in the benchmarking of our performances guarantees the reliable identification of new biological marker through integration with molecular profiles beyond the limits of single experimental series [11].

For the future improvement and long term development of archival procedures and facilities, we are currently preparing an integration of our efforts with the "HoriaCernescu-Research Unit" of the University of Agricultural Sciences and Veterinary Medicine of Banat "King Michael the 1st of Romania", Timisoara. While the infrastructure of this facility so far has been used for the collection of non-human samples, making use of the expertise and existing infrastructure of both institutions would significantly expand our scope, and potentially be instrumental in future projects utilising distinct animal models in pre-clinical human rare disease research.

Innovation (innovative solutions for any kind of biobank-related problems)

Our biobank, consisting of nucleic acids, corresponding blood and tissue samples accompanied by detailed long term follow up data, is the first dermatologic samples bank in Romania. This institution in conjunction with the national and international collaborations present a Multidisciplinary Research Core Unit at our university, in which medical doctors, graduate and undergraduate students of different professions are directly involved in clinical research and project development. The senior scientists with internationally recognized research background provide the high quality scientific milieu required. Our biobank in conjunction with all the resulting scientific research efforts around, is providing the international guarantee for increased Romanian research visibility, sustaining the projects' development towards

an increased dissemination of obtained results within the international scientific community [12].

Sustainability (outline of business model)

The biobank is currently linked to the infrastructure of the Victor-Babes University of Pharmacy and Medicine Timisoara and is financed by the end of 2015 by the Romanian-Swiss Research Programme (RSRP; research grant SNF142305).

By the end of this year, we plan to reconstitute the biobank as an independent institution upon joining with the facilities for non-human research of the "HoriaCernescu Research Unit". Sustained financing would be ensured by national grants linked to the development of the medical infrastructure in Romania. As we envision however becoming a nation-wide center of competence we currently discuss the possibility to sub-contracting as non-profit "service providers" with individual research projects in which bio-banking expertise and data is required. This way we would also raise interest for our institution within other hospitals throughout the country and ensure a standardized protocol and sustained quality for human medical research [13].

This study was performed respecting all the international ethical guidelines for human biomedical research . All the samples in our biobank were collected with full donor authorisation based on sufficient information and adequate understanding of both the proposed research and the implications of participation in it .

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