



Integrated Project „Novel molecular diagnostic tools for the prevention and diagnosis of pancreatic cancer (MolDiag-Paca)”

Executive Summary

1st reporting period; Aug 1st 2006 – July 31st 2007

Malignant tumours of the pancreas, known as pancreatic carcinomas, remain among the most serious challenges in modern medicine. Although not among the most common tumours, they are among the most frequent causes of cancer-related deaths, with approximately 28,000 deaths per year in the USA and 40,000 per year in Europe. There are currently no means for the reliable diagnosis of early stages and for curative treatment of late stages of the tumour.

The overall aim of this EU Framework Programme 6 Integrated Project (“MolDiagPaca”; visit our project website at www.moldiagpaca.eu) is to make use of genetic profiles of pancreatic cancer and precursor lesions to improve the outcome of pancreatic cancer patients by providing novel and highly efficient molecular diagnostic tools for an early diagnosis. One of the major prerequisites in order to achieve this ambitious aim is an integrated multidisciplinary research approach, which enables a strong interaction between technology, biology and medicine to translate genome data into practical, clinical applications.

EU funding for this consortium was started August 2006 for three years, bringing together 19 European partners from academia and industry with long-standing interest in pancreatic cancer biology, diagnosis and treatment (see also www.moldiagpaca.eu). Since the express goal of this consortium is to generate molecular diagnostic tools that will be ready for clinical applications versus the end of the project, inclusion of Small and Medium sized Enterprises (SME) with a particular interest in developing molecular diagnostic tools was a special priority from the beginning.

During the first reporting period (months 1-12), the consortium laid the fundamental prerequisites for the final project goals (development of molecular tools for early diagnosis of pancreatic cancer). In summary that means that resources (i.e. tissue, serum, plasma, urine, DNA, RNA, tissue arrays) were collected, databases were generated, lists of genes were generated as well as protocols optimised, and assays established.

Among several databases established by contractors P2* and P5, a database of individuals from hereditary pancreatitis families or families with multiple cases of pancreatic cancer was generated within workpackage (WP) 1. These databases, which were generated with the input from partners P1, P2, P5, P7, P11, P13 and P18, will help to understand and detect the determinants of risk towards hereditary pancreatitis and pancreatic cancer. Among the mutations which are suspected to be causative for pancreatic cancer development are mutations in the DNA repair gene BRCA2. In order to further investigate this hypothesis, assays were established by P2 to identify BRCA2 mutation carriers. BRCA2 heterozygotes are identified with comet and micronuclei assays (Fig. 1).

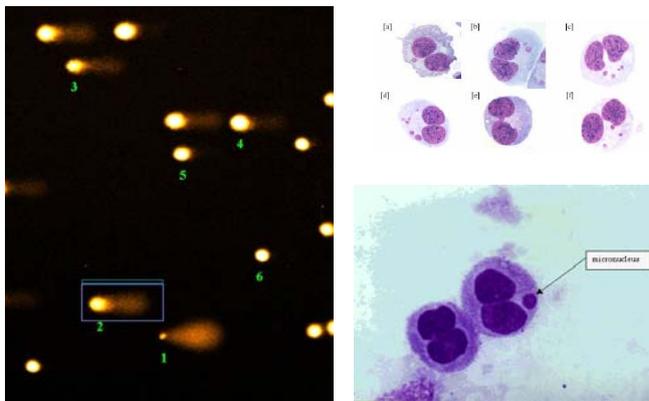


Fig. 1: Examples of comet assays (left) and micronuclei assays (right) to uncover DNA repair defects.

With respect to sporadic cases of pancreatic cancer, a database was generated by P3 during the first 12 months containing data on genome, transcriptome and proteome profiles of preneoplastic lesions, early and advanced ductal tumours and other exocrine tumours, as well as prognostic and therapeutic tumour subgroups identified from analysis of the ESPAC1 and ESPAC3 (European Study Group for Pancreatic Cancer) clinical trials of chemotherapy in pancreatic cancer. Transcript profiles of additional tumour entities, i.e. endocrine tumours and rare exocrine tumours, are currently being generated within the consortium (P1, P2, P4, P7). This database will constantly be updated and can be accessed by all partners in the IP to select individual target genes or signatures suitable to develop molecular diagnostic and prognostic tools. Moreover, it will help to generate a comprehensive picture of molecular aberrations present in advanced tumours as well as, more importantly, early tumour stages and precursor lesions. This knowledge will be indispensable for the development of urgently needed novel approaches for the early diagnosis of pancreatic cancer.

One example for the development of novel diagnostic tools developed on the basis of the molecular data accumulated in the consortium's databases and the resources collected and

* For Numbering of MolDiagPaca participants see last page

provided by P2 is a specialised diagnostic cDNA array which is currently being developed by P1. Protocols for the extraction of minimal amounts of RNA from clinical materials such as fine needle aspirates, pancreatic juice, circulating tumour cells, or brush cytologies have been established and validated. Furthermore, a set of relevant marker genes for differential diagnosis has been selected, and the array's diagnostic performance is currently being evaluated.

In a complementary approach, P2 and P3 are conducting quantitative and qualitative advanced proteomic analyses of serum, pancreatic juice and urine in search of biomarkers that could be useful potentially as diagnostic markers of pancreatic cancer. Prerequisite to this aim was the standardised protocol for MDLC-MS analysis of serum which was developed during the first 10 months by P2. Moreover, protocols were developed by P3 to efficiently perform multiplexed liquid chromatography MS (iTRAQ) on patient sera. In the upcoming project phase, further proteomic-based methods, based on MDLC, iTRAQ, SELDI-TOF, and 2D-DIGE, will be applied to the analysis of patient material, and, the best combination of diagnostic markers will be selected for the development of a standardised diagnostic procedure.

Another focus of the consortium lies in the characterisation of pancreatic cancer-specific changes in DNA methylation (P4). The identification of candidate loci for pancreatic cancer-specific epigenetic misregulation by analysis of the available literature and the data from the clinical partners (P1, P2, P5, P7, P8, P11, P13) is the first step towards the development of a complete diagnostic tool. Thus, during this first period, ca. 500 genes were defined, i.e. analysed in detail with respect to methylation variations in their promoter regions and the first exon. Furthermore, levels of promoter methylation in DNA from pancreatic juice samples were measured and compared to levels in matched tissue and other markers (e.g. p53 and K-Ras mutation) in juice and tissue, and a functional array established (Fig. 2). Both, primary samples taken from tumour biopsies or tissue samples, as well as serum samples were analysed.

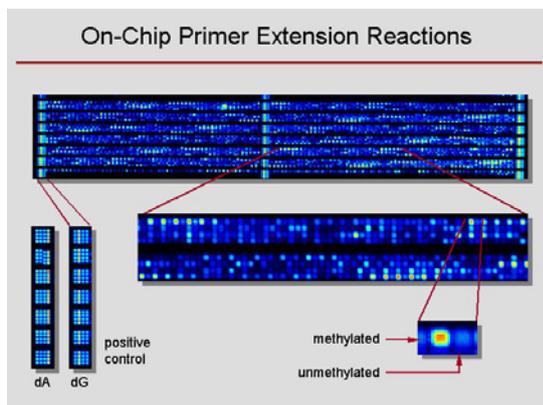


Fig. 2. Example of an on-chip primer extension reaction for epigenetic profiling.

A great hope for significant advancements in the early diagnosis of pancreatic ductal adenocarcinoma (PDAC) and its precursor lesions (PanINs) lies in the development of novel molecular imaging approaches based on the *in vivo* detection of cancer-specific single molecular markers identified in high-throughput approaches. The development of such molecular imaging techniques and their evaluation in animal models is one of the ambitious aims of this consortium. In a first step, 15-30 diagnostic possible targets (candidate genes) that are over-expressed in PDAC and/or PanINs have been already selected (by P1, P3, P4, P8, P10, P13, P15, P19, P20). Currently, specific antibodies for the validation of candidate proteins and a list of candidate proteins validated in primary tissue are being provided. These proteins will be detected *in vivo* by different imaging reagents and techniques, which will primarily be validated in murine xenograft models. Ultimately, clinical applications of this *in vivo* imaging tool could enable early diagnosis of PDAC at a curable stage of the disease.

Fundamental to the overall success of the consortium will be the evaluation of diagnostic procedures in a clinical setting. In order to achieve its ambitious goals, the consortium will take a comprehensive approach, evaluating the suitability and applicability of diagnostic tools (both those already existing and those currently being developed by partners [P1, P2, P7a, P7b, P8, P11, P14, P18] in the IP) in specimens from patients with a familial risk of developing pancreatic cancer. Two already existing registries, EUROPAC (The European Registry Of Hereditary Pancreatitis And Familial Pancreatic Cancer) and FaPaCa (Familial Pancreatic Cancer) assist this plan and are being extended in return by the IP. To date, 94 high risk individuals have been recruited for screening. Furthermore, ethical approval (MREC) for obtaining additional (follow-up) samples from ESPAC patients has been obtained. In addition, we will also make comprehensive use of the samples arising from the ESPAC trials to further characterise the existing diagnostic markers and those arising within the consortium. Detection of novel markers and changes in their levels will be correlated with 1. type of pancreatic malignancy 2. stage and grade of tumour 3. metastatic disease. 4. response to treatment and 5. disease progression. This will provide an overall picture of the possible application of these markers in a clinical setting.

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P7 – University of Verona, Italy

P8 – University of Bochum, Germany

P10 – Schering (Bayer Schering Pharma), Germany

P11 – University of Heidelberg, Germany

P12 – Cyclacel Ltd., Dundee, UK

P13 – Universitätsklinikum Schleswig-Holstein, Germany

P14 – Tepnel Life Sciences PLC

P15 – University of Münster, Germany

P16 – Advalytix AG, Germany

P17 – Asper Biotech, Estonia

P19 - Forschungsverbund Berlin (FMP), Germany

P20 – Universität Ulm, Germany