

## Massively Parallel Sequencing Analysis of Genetic Alterations Carried by Pancreatic Adenocarcinoma

Marina Macchini<sup>1</sup>, Annalisa Astolfi<sup>2</sup>, Riccardo Casadei<sup>3</sup>, Valentina Indio<sup>2</sup>, Silvia Vecchiarelli<sup>1</sup>, Claudio Ricci<sup>3</sup>, Marielda D'Ambra<sup>3</sup>, Elisa Grassi<sup>1</sup>, Donatella Santini<sup>4</sup>, Carla Serra<sup>5</sup>, Raffaele Pezzilli<sup>5</sup>, Francesco Minni<sup>3</sup>, Guido Biasco<sup>1</sup>, Mariacristina di Marco<sup>1</sup>

<sup>1</sup>Department of Oncology, <sup>2</sup>“G. Prodi” Cancer Research Center, <sup>3</sup>Department of Surgery, <sup>4</sup>Department of Pathology, <sup>5</sup>Department of Internal Medicine; Sant’Orsola-Malpighi Hospital. Bologna, Italy

**Context** Pancreatic cancer (PC) is characterized by a 5-year survival rate of 4%. The molecular mechanisms involved in the high tumorigenicity of PC are not yet well-known. **Methods** PC samples from 7 localized and advanced cases of pancreatic adenocarcinomas were collected by ultrasound-guided biopsy. Whole transcriptome RNA libraries were sequenced at 75x2 bp on a HiScanSQ Illumina platform. An average of 4.5x10<sup>7</sup> reads per sample were generated, with a mean read depth of 40. Sequences were mapped to the human genome (build hg19) and the single nucleotide variants (SNVs) were detected with SNVMix2 tool. The SNVs were compared with genetic variations databanks (dbSNP, 1000genomes, Cosmic) in order to highlight the novel variants. The non-synonymous SNVs were considered at the protein level and analyzed with SNPs&GO, in order to predict whether a variation is disease-related or neutral. **Results** Pancreatic adenocarcinomas exhibited a mean of 139 (range: 65-252) non-synonymous SNVs, of which 12

on average are potentially disease-related. We found mutations in oncogenes and tumor suppressor genes known to be related to pancreatic tumors, as 4/7 patients exhibited KRAS oncogenic variants (p.G12D, R or V) two of which carried also mutations either in SMAD4 or PIK3CA, one patient showed both TP53 and CDKN2A inactivating mutations and one a mutation in SMARCA4. Novel disease-related SNVs were found in genes regulating cell cycle progression and proliferation, apoptosis, TGFbeta and integrin-signaling, epithelial to mesenchymal transition and nucleotide excision repair. Of interest some genes were mutated in multiple patients, as CBLC that regulates intracellular signalling by multiple tyrosine kinase genes. **Conclusions** Next generation sequencing analysis, through the identification of the oncogenic alterations carried by tumor cells, can improve the understanding of pancreatic carcinogenesis and highlight new biomarkers for early diagnosis and potential therapeutic targets in pancreatic cancer.