Hot topics in epigenetic regulation of cancer self-renewal for pancreatic tumors: future trends

Ramon Andrade De Mello^{*1,2,3,4}, Inês Faleiro^{1,4}, Joana D Apolónio^{1,4}, Uri Tabori⁵, Aryeh J Price^{5,6}, Vânia P Roberto^{1,4} & Pedro Castelo-Branco^{1,4}

¹Department of Biomedical Sciences & Medicine, Algarve Biomedical Center, Division of Oncology, University of Algarve, 8005-139 Faro, Portugal

²Faculty of Medicine, University of Porto, 4200-319, Oporto, Portugal

³Division of Medical Oncology, Bauru State Hospital, & School of Medicine, Universidade Nove de Julho (UNINOVE), 17011-102, Bauru, São Paulo, Brazil

⁴Centre for Biomedical Research (CBMR), University of Algarve, 8005-139 Faro, Portugal

⁵Arthur & Sonia Labatt Brain Tumor Research Center, The Hospital for Sick Children, University of Toronto, Toronto, M5G 1X8, ON, Canada

⁶Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA

*Author for correspondence: ramondemello@gmail.com

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Self-renewal is critical in order for a cancer to proliferate, grow and eventually metastasize. Today, several molecular mechanisms are being studied, which may explain the emergence of this property in advanced cancer. Pancreatic cancer is both a deadly and highly recurrent tumor, relying heavily on accelerated proliferation. Understanding self-renewal in this tumor type is therefore likely to prove essential for reaching better disease control. Recent advances in our understanding of telomere maintenance pathways are beginning to yield those insights [1]. Indeed, it is now known that telomerase reactivation in cancer is intimately related with expression of the *TERT* gene. The multimodal regulation of this locus, along with transcriptional output itself, both serve as prognostic factors across a variety of tumor types.

In 2006, Clément *et al.* analyzed early molecular markers of malignant transformation that have the potential for clinical implementation [2]. Gene promoter methylation analysis was performed on normal, preneoplastic and neoplastic lesions from Barrett's esophagus (BE) patients. The *TERT* promoter was hypermethylated in 100, 91 and 92% of cases, whereas in BE mucosa from patients who had not progressed (16 samples) methylation was found only in 36, 23 and 17%, respectively. These data suggested that promoter methylation profiling of BE using multiple target genes including *APC*, *TIMP3* and *TERT* could be used as a predictive panel for increased esophageal adenocarcinoma susceptibility [2].

In 2013, Castelo-Branco *et al.* suggested that hypermethylation of a specific region in the *TERT* promoter is associated with *TERT* expression. The signature discovered is predictive of tumor progression and poor prognosis in pediatric brain tumors. This differentially methylated region is remarkably accessible, given that it is easy to amplify, and readily assayed using standard clinical laboratory reagents [3]. Later studies have supported pan-cancer significance for this biomarker, including a correlation with biochemical recurrence in prostate cancer [4] and diagnostic capacity in neuroblastoma [5].

Pancreatic cancer proved to be an excellent application for this new biomarker. Recently, we analyzed data retrieved from the The Cancer Genome Atlas (TCGA; <http://xena.ucsc.edu/>) on 193 patients with pancreatic adenocarcinoma [1]. Exclusion criteria included history of neoadjuvant radiotherapy or chemotherapy as those

parameters could independently influence methylation levels. We used the Illumina Infinium HumanMethylation450K array to analyze this cohort (normal tissue [n = 10] and primary tumor [n = 183]). We found the CpG site targeted by the probe cg11625005 (chr5:1,295,737; position -575 in relation to transcription start site), localized within the *TERT* hypermethylated oncologic region (THOR; -591/-159), in pancreatic adenocarcinoma. Since THOR was shown to associate with *TERT* expression [3], our group investigated *TERT* expression in pancreatic cancer and found a positive correlation with THOR methylation levels. In addition, THOR could differentiate normal tissue from stage I to IV of the disease supporting its use as an early disease biomarker.

Currently, advanced pancreatic cancer approaches are very limited in terms of overall survival benefits [6]. Finding a novel biomarker for tailoring treatment is an urgent unmet clinical need. Extant biomarkers are generally classical precursors of pancreatic cancer, such as *KRAS* mutations and inactivation of tumor suppressor genes (*TP53*, *p16/CDKN2A* and *SMAD4*) [7,8]. Improving on the status quo and finding meaningful biomarkers will require developing a deep understanding of pancreatic cancer pathogenesis. THOR is one such example. Highly methylated THOR correlates with overall and recurrence-free survival of the patients suggesting that THOR can help predict patient outcome. In borderline resectable disease, we hypothesize that THOR data could help clinicians decide whether or not to indicate chemotherapy followed by radiation and then surgery or refer the patient for a clinical trial. In selected patients with ECOG performance status 2 due to heavy tumor load, THOR levels might identify the optimal chemotherapy regimen for achieving a response. There are many more clinical prospects for THOR, and biomarkers in its class; however, larger cohorts and randomized Phase III trials will be necessary.

Furthermore, circulating tumors cells (CTCs) faces an interesting scenario in the clinical practice [9]. Today, several platforms and methods have been described to detect and isolate CTCs from the human blood [9–11]. The liquid biopsy, the detection and enumerating of CTCs, is considered a very practical and useful clinical tool to monitoring treatment responses and estimate the probability of tumor relapse. Also, it is an important noninvasive tool for molecular characterization and tumor biomarkers analysis without need of tumor tissue biopsies. In 2015, Salanti *et al.* reported an interesting research [12], which showed the role of VAR2CSA malaria protein for the oncofetal chondroitin sulfate (ofCS) detection, a highly specific target for CTCs isolation. The ofCS is expressed by placental and cancer cells of both epithelial and mesenchymal origin [13]. The authors [12] suggested that ofCS could play a key role in tumor-cell motility through integrin signaling pathways, and therefore supports the metastatic potential of cancer cells [13]. Testing a wide range of cells and tissues, the authors found that the recombinant VAR2CSA protein binds >95% of cancer cell lines and tissues of epithelial, mesenchymal and hematopoietic origin, with very limited binding to noncancerous cells or normal tissue (besides placental tissue), which it could improve its role as tool to broadly and efficiently capture rare cancer cells in complex blood samples [10,12]. Liquid biopsy is approved by the US FDA for the EGFR analysis in lung cancer patients [14]. Currently, the CellSearch™ platform is the only gold standard and approved by FDA for all the CTCs detection strategies [14]. In addition, methylation analysis of circulating tumor DNA could be useful to determination of the primary cancer location with a specificity and sensitivity of 99.6 and 97.7%, respectively [15,16]. It would be a very helpful tool in the near future to characterize pancreatic cancer molecular profile in order to help clinicians in terms of personalized medicine. Thus, more robust and well-designed clinical trials are important to assess the role of all these aforementioned biomarkers in the pancreatic cancer framework.

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