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Titolo	USE OF NEXT-GENERATION SEQUENCING TO STUDY CODING AND NON-CODING RNA IN COLORECTAL CANCER [Tesi di dottorato]
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Sommario	The identification of novel mRNA and small RNA signatures of prognostic and diagnostic value in colorectal cancer (CRC) is primary focus of the thesis. The overall aim of the body this work is a deeper understanding of the molecular causes in the pathology of CRC and the identification of biomarkers, specifically mRNAs and other small non-coding RNAs with prognostic values in the clinical setting. These findings would in turn lead to an optimization of the therapeutic targets and ultimately to better clinical management of patients diagnosed with CRC. Next-generation sequencing (NGS) is based on deep sequencing, which produces billions of short sequences at a time. NGS benefits biomedical research in several ways by interrogating whole or partially targeted genomes, transcriptomes and epigenomes, including non-coding RNAs (ncRNAs) and microRNAs (miRs). NGS is able to rapidly generate large amounts of sequence data at substantially lower cost and time respect Sanger Sequencing. I have been involved in the development and application of various novel techniques for the construction of sample libraries for NGS analysis. I have also worked with various methods of analysis of next-generation sequencing data of cancer samples. In addition to NGS, I have also worked with numerous genomics technologies including, microarrays (both commercial and custom), NanoString, Real-Time PCR, protein arrays, and other genomics technologies to investigate not only colorectal cancer, but

	several other types of cancer including, but not limited to leukemia/lymphoma, breast cancer, head/neck cancer, osteosarcoma, and lung cancer. MicroRNAs are non-coding RNA regulators of protein output by way of coding RNA disruption. MicroRNAs have been shown to be differentially expressed in many solid cancers, and they can be considered biomarkers for predictive signatures in cancer. The effects of microRNAs are exerted on cell pathology and physiology controlling translation of tens or even hundreds of different coding messengers and a unique messenger can be controlled by more than one microRNA. In turn, one, or more, microRNAs, can disrupt entire physiological pathways. Predictive markers are important in oncology as tumors of the same tissue of origin vary widely in their response to most available systemic therapies. Of all human cancers, colorectal cancer (CRC) is the third most commonly diagnosed cancer in the world at more than 500,000 new cases diagnosed per year. Currently, the Tumor-Node-Metastasis (TNM) is currently the most effective and reliable predictor of CRC outcomes. However, recently new genetic alterations have been uncovered which could potentially be used to estimate prognosis in CRC, with several of them potentially representing predictive markers towards appropriate treatment regimens. Unfortunately, most of these biomarkers have failed validation in the clinical setting, with some notable exceptions being loss-of-function mutations in KRAS, BRAF, SMAD4 and TP53. In addition, there are genetic alterations such as chromosomal instability (CIN), loss of heterozygosity (LOH), micro-satellite instability (MSI), that affect mismatch repair (MMR) genes, including hMLH1, hMSH2, hMSH6, and PMS2. The overall predictive values of CIN and MSI remain controversial and the role of influence from mutations in other key genes involved in carcinogenesis still largely unclear. Short RNAs were sequenced from paired colon adenocarcinoma and normal samples. The RNA sequences were aligned on the human genome by using multiple independent algorithms. All short RNA sequences were de novo merged into more than 250,000 distinct RNA contigs covering the human genome. These de novo short RNA contigs, or shortigs, were then matched to human genome annotation. Using this unbiased genome wide approach, all short RNAs were profiled in colon adenocarcinoma. Alongside known miRNA, snoRNAs, piRNA, over 60 RNAs were differentially expressed from non annotated shortigs, and represented candidates for novel cancer non-coding genes. RNA expression plots were obtained for each shortig, revealing RNA processing of precursor miRNAs or even of entire miRNA clusters. A number of discrepancies with miRBase annotations were detected. The dynamic range and specificity of next generation sequencing allowed an unprecedented insight into miRNA and other non-coding RNA expression in colorectal cancer.
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