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Non-coding RNAs: More Questions than Answers

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The sequencing of the human genome marked not the end of the genomics field, but rather its beginning. After more than a decade since the first human genome was sequenced, annotated and published, researchers still have a long journey ahead to understand the meaning of all the information encoded in human's DNA. This is mainly because there are other layers of information beyond the genome, for example, the epigenome and the transcriptome. Genomics and genetics mainly study the differences between genomes and gene expression changes. Transcriptomics studies the whole set of transcripts produced by a specific cell type at any given time. Finally, the epigenome is able to control the expression of different genes by various mechanisms. Importantly, there is a strong dynamic between the "static" genome, represented by the DNA molecule, the transcriptome, representing the transcripts that are expressed, and the epigenome, regulating all the information transacted between the genome and the transcriptome. Recently, new classes of transcripts with no protein-coding capacity - non-coding RNAs (ncRNAs) - have been described as part of the human transcriptome. The question that researchers are facing now is if the majority of these transcripts are functional. In this article, we provide some proofs that the majority, if not all, have a function in human cells and will have a big impact for cancer research.

With the advent of new technologies to sequence DNA, such as second- and third- generation DNA sequencers [1], it is becoming clear that human genomes transcribe more than the approximately 3% of protein-coding regions (human genomes have an estimate of 25,000 protein-coding genes according to the Human Genome Project that finished in 2001) [2]. Depending on the technology used, up to 90% of transcripts representing a genome can be transcribed into RNA [3]. This fact has prompted researchers to re-evaluate the definition of a "gene" [4]. Genes are not seen anymore as mere linear stretches of DNA that are transcribed and translated into proteins. The most recent definition of a gene suggests that they represent a union of genomic sequences encoding a coherent set of potentially overlapping functional products [5,6]. The genomic sequences encoding functional products can be sense or anti-sense and produce RNA transcripts in different ways, thus increasing the complexity of the human transcriptome.

Although initially described as "transcriptional noise" or accumulated evolutionary debris arising from the early assembly of genes and/or the insertion of mobile genetic elements, recent evidence suggests that transcriptional units with no coding capacity or ncRNAs, may play major biological roles in cellular development, physiology and pathologies. There is also accumulating evidence that the majority of ncRNAs might be implicated in epigenetic mechanisms of gene regulation [7]. Therefore, big challenges lie ahead in order to understand the transcriptome of cells in both normal and pathological conditions. In that regard, a field of increasing interest that may provide clues to this conundrum in genomic science is the emerging field of epigenomics.

The epigenome is defined as the group of modifications that can occur at the genomic level that will not change the sequence of the bases of the DNA, but can change the DNA conformation and, as a consequence, change gene expression [8]. Epigenetics is the study of these modifications in single genes and/or groups of genes. The main epigenetic modifications that can occur in the DNA molecule are: 1) binding of different proteins that can act as chromatin insulator elements or repressors to the DNA such as histones, methyl-binding proteins, polycombs and others; 2) addition of chemical groups in the bases of the DNA such as methyl groups (CH_3), which are typically added to the position 5 of the pyrimidine ring of cytosine residues that are located in CpG dinucleotides [9] (however other types of chemical modifications may also occur) and 3) microRNAs (miRNAs) and other non-coding RNAs that can regulate the expression of other genes by base-pairing to mRNAs and/or by physically binding to the DNA. Epigenomics has recently become an important field after the Human Epigenome Project (HEP) was launched in the United States and in Europe. The main aim of this project is to identify, catalogue and interpret genome-wide DNA methylation and histone modification patterns of all human genes in major tissues. As discussed, while epigenetics refers to the study of single genes or groups of genes, epigenomics refers to wider and global analyses of epigenetic changes across entire genomes.

One could argue that most ncRNAs are non-functional but, in recent years, it became clear that non-protein coding RNAs or ncRNAs are an important part of the transcriptional output of mammals and other complex organisms such as humans [10]. Indeed the eukaryotic genome, rather than being viewed as an island of protein-coding genes in an expanding sea of evolutionary "junk" DNA, may be better thought of as an RNA machine since the majority of genomic DNA is transcribed as described above [11]. The best-studied ncRNA group are miRNAs. miRNAs are small, evolutionarily conserved, non-coding RNAs of 18-25 nucleotides in length that act as expression regulators of genes involved in development, cell differentiation, proliferation, survival and death [12]. The specificity of miRNA targeting is defined by Watson-Crick complementarity between positions 2 to 8 from the 5' miRNA with the 3' untranslated region (UTR) of their target mRNAs (even though exceptions to this rule may occur) [13]. Each miRNA has the potential to target from hundreds to thousands of other genes and

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can also activate or inhibit gene expression by different mechanisms [14]. In addition, deregulated expression of miRNAs has been extensively described in a variety of diseases, especially in cancer [15].

More than 1,500 miRNAs have been identified (by experimental analyses and bioinformatics predictions) and characterized in the human genome. miRNAs have become an important area for cancer research since several studies have already shown deregulated expression of miRNAs and their targets in different tumor types [15]. Thus, a better understanding on the mechanisms that miRNAs use to control gene expression will help in: 1) identifying functional miRNA gene targets (that could number from hundreds to thousands) and will provide a larger picture on how cancer cells transform, multiply, and invade surrounding tissues in tumors; 2) identifying the clinical conditions under which miRNAs will be useful markers for early diagnosis (exemplified by circulating miRNAs in the blood and body fluids of cancer patients that have been published), to improve both prognosis and treatment; and 3) defining the best conditions for the use of miRNAs and their inhibitors as anti-cancer agents in translational medicine by identifying which patients will benefit from these therapies. Clinical trials for cancer using discoveries in this area are showing promising results; however we need a better understanding of miRNA functions since their use as therapeutic agents can perturb entire networks of proteins.

Other classes of ncRNAs, differing in size and function, were also identified in the human genome. Examples include Long Intergenic RNAs (or LincRNAs) that are closely associated to epigenetic mechanisms [16]. LincRNAs range in size from hundreds to tens of thousands of bases with several of these already identified in the human genome [16]. LincRNAs also have deregulated expression in tumors [17] and can be used as cancer biomarkers [18]. Additionally, there are small RNAs [19], anti-sense transcripts [20] and others that are implicated in different types of cancer. Since the functional examples of these new classes of ncRNAs are just starting to grow in the literature, there is much skepticism about the importance of these newcomers. As discussed, many miRNAs regulate pathways in cancer as part of a genetic and epigenetic network and have been used to develop diagnostic, prognostic and therapeutic strategies; however we are just starting to envision the use of other types of ncRNAs with the same purposes.

It is a fact that most of the new classes of ncRNAs identified in the human transcriptome by different studies have unknown functions. However, there are many lines of evidence suggesting that these RNAs are biologically significant. Understanding the functions of these new classes of ncRNAs in normal mechanisms and in diseases such as cancer will be a big challenge. Even though miRNAs have defined functions, other types of ncRNAs (exemplified by small and long RNAs) need more experimental evidence of functionality. Studies showing the association of long ncNRAs and epigenetics are increasing in literature, and we believe that most ncRNAs will play important roles in the epigenome. If this is the case, ncRNA expression and regulation will have a great impact in cancer research. The number of publications about ncRNAs and oncology is rapidly growing, and this fact suggests that ncRNAs may be more important than previously thought. However, we still have more questions than answers in this evolving field of study applied to oncology. Even with a lot of questions without answers, it is now clear that cancer researchers will be occupied in the years to come trying to understand the functions of these new players in the epigenome.

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