PORZIONE LETTA: N°2

Leading Edge
Review

Cell

The Role of Non-coding RNAs in Oncology

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For decades, research into cancer biology focused on the involvement of protein-coding genes. Only recently was it discovered that an entire class of molecules, termed non-coding RNA (ncRNA), plays key regulatory roles in shaping cellular activity. An explosion of studies into ncRNA biology has since shown that they represent a diverse and prevalent group of RNAs, including both oncogenic molecules and those that work in a tumor suppressive manner. As a result, hundreds of cancer-focused clinical trials involving ncRNAs as novel biomarkers or therapies have begun and these are likely just the beginning.

Although now one of the hottest topics in biomedical science, the importance of non-coding RNA (ncRNA) was largely unrecognized until recently. RNA was once thought to mostly serve as a messenger that carried instructions encoded in DNA so that other molecules, like the ribosome, could use the code to make proteins. However, in the last 30 years, researchers have discovered that multiple types of RNA exist, and among the most important is ncRNA—the type that is not involved in producing proteins. The discovery of tens of thousands of ncRNA species has revolutionized the field, altering the way that researchers think about physiology and the development of disease (Adams et al., 2017; Bartel, 2018; Evans et al., 2016; Rupaimoole and Slack, 2017). ncRNAs constitute more than 90% of the RNAs made from the human genome, but most of the >50,000 known ncRNAs have been discovered only in the past 10 years and remain largely unstudied (Deveson et al., 2017; Esposito et al., 2019; Kopp and Mendell, 2018; Ransohoff et al., 2018).

Still, there are many ncRNAs that have since been shown to play key roles in both normal cellular function and disease, including cancer, and this information is being actively translated into the clinic. Some small ncRNAs are so stable that they survive in the bloodstream and could be the basis for accurate and sensitive screens for major human cancers in a few drops of blood (Yaman Agaoglu et al., 2011; Imaoka et al., 2016; Toiyama et al., 2013). Additionally, ncRNAs can be therapeutically targeted, and the delivery of ncRNAs can be based on an existing foundation of what has been learned regarding delivery of RNAi and oligonucleotides targeting protein-coding mRNAs (Levin, 2019; Pecot et al., 2011; Wu et al., 2014). In fact, the field of RNA medicine has seen a renaissance (Levin, 2019) with the recent approval of the first RNAi drug Onpattro (patisiran; reduces levels of *TTR* for treatment of the neurodegenerative disease hereditary transthyretin amyloidosis) (Adams et al., 2018) and the clinical success of the RNA-targeting oligonucleotide drug Spinraza (nusinersen; increases levels of full-length *SMN2* for treatment of the neuromuscular disease spinal muscular atrophy) (Wurster et al., 2019). In addition, clinical trials with drugs based on a class of ncRNAs called microRNA (miRNA), either therapies that increase or decrease the target miRNA, have begun for cancer (Beg et al., 2017; Seto et al., 2018; van Zandwijk et al., 2017).

In this review, we will discuss ncRNAs in relation to cancer cell biology and their relevance to current clinical practice. We first examine the intricacies of the different classes of ncRNAs (miRNAs, transfer RNA [tRNA]-derived small RNAs [tsRNAs], PIWI-interacting RNAs [piRNAs], long ncRNAs [lncRNAs], pseudogenes, and circular RNAs [circRNAs]) (Table S1) and provide fundamental examples of the far-reaching roles that these molecules have in affecting cancer processes. We then discuss how these basic science insights in ncRNA biology are being used to develop next-generation diagnostics and therapies in cancer. As the so-called "dark matter" of the genome continues to be brought into the light, it is evident that targeting ncRNA signaling has great potential to impact cancer patient care.

Overview of Classes of ncRNAs and Their Association with Cancer

For decades, the miniscule protein-coding portion of the genome was the primary focus of medical research. The sequencing of the human genome showed that only ~2% of our genes ultimately code for proteins, and many in the scientific community believed that the remaining 98% was simply non-functional "junk" (Mattick and Makunin, 2006; Slack, 2006). However, the ENCODE project revealed that the non-protein coding portion of the genome is copied into thousands of RNA molecules (Djebali et al., 2012; Gerstein et al., 2012) that not only regulate fundamental biological processes such as growth,

development, and organ function, but also appear to play a critical role in the whole spectrum of human disease, notably cancer (for recent reviews, see Adams et al., 2017; Deveson et al., 2017; Rupaimoole and Slack, 2017). Trailblazing research led to our understanding of how ncRNA molecules perform multiple vital roles in the coding, decoding, regulation, and expression of genes as well as how they communicate with each other (Anastasiadou et al., 2018b; Esquela-Kerscher and Slack, 2006; Gregory and Shiekhattar, 2005; Huarte and Rinn, 2010; Kasinski and Slack, 2011; Krichevsky et al., 2003; Rinn and Huarte, 2011; Tay et al., 2014). This knowledge of ncRNA regulatory roles revealed specific ncRNA networks based on complementary base pairing at work in different cancer types (Anastasiadou et al., 2018b), which in turn, opened up possibilities for scientists in this field to develop specific cancer therapeutic and preventive strategies focused on the ncRNAs made from the human genome (for recent reviews, see Cieślik and Chinnaiyan, 2018; Rupaimoole and Slack, 2017).

Cancer is characterized by cells that grow (proliferate) out of control, are able to spread to other tissues (metastasize), and lose the ability to die through the orderly process of cell death (apoptosis). The discovery of ncRNA has added a new dimension to the understanding of how cancer develops, and how it may be treated, by providing a window into the impact of the rest of the genome. Deregulated ncRNA expression, and subsequent downstream signaling processes that we detail in later sections, have been directly implicated in cancer development and progression. Genetic alterations in genes encoding ncRNAs have been found to be associated with cancer; however, compared to protein-coding genes, the list of genetic examples from studies thus far is considerably shorter. The most notable example is likely deletion of 13q14.3 in chronic lymphocytic leukemia (CLL) that deletes the miR-15/16 tumor suppressors (Calin et al., 2002). Conversely, amplification of chromosomal regions encoding oncogenic ncRNAs are also found in cancer, including amplification of lncRNAs *FAL1* (Hu et al., 2014) and *PVT1* (Tseng et al., 2014). Single nucleotide polymorphisms (SNPs) in the genes of lncRNAs *H19* (Hua et al., 2016), *ANRIL* (Pasmant et al., 2011), and *CCAT2* (Ling et al., 2013) have also been associated with varying risks of cancer development. In addition to genetic alterations within transcribed regions, mutations in the promoters of ncRNAs can lead to altered gene expression levels, such as recurrent driver mutations in the promoters of lncRNAs *NEAT1* and *RMRP* in breast cancer (Rheinbay et al., 2017). Besides aberrations in sequences encoding the ncRNA itself, mutations or dysregulation of enzymes involved in the biogenesis of ncRNAs are implicated in cancer, such as Drosha and Dicer involved in miRNA processing (Rupaimoole and Slack, 2017). In addition to these genetic mechanisms, up- or downregulation of ncRNA expression associated with cancer can occur through epigenetic, transcriptional, or post-transcriptional processes (see recent reviews Adams et al., 2014; Anastasiadou et al., 2018a; Rupaimoole and Slack, 2017).

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ncRNAs can be divided into different classes, broadly based upon their size. The small ncRNAs important in cancer include miRNAs, tsRNAs, and piRNAs. At the opposite end of the size spectrum are the lncRNAs, which are characterized as untranslated RNAs greater than 200 nt in length, and include subclasses such as pseudogenes and circRNAs.

MicroRNAs

Near the turn of the millennium, the first miRNAs, *lin-4* and *let-7*, were identified through developmental studies in *C. elegans* (Lee et al., 1993; Reinhart et al., 2000). miRNAs are short ncRNAs of ~22 nt in length that regulate the expression of other RNAs, notably mRNAs through binding between the 5′ end (known as the "seed") of the miRNA with complementary sequences in target RNAs. Genes encoding miRNAs are transcribed by RNA polymerase II (Pol II) and processed through an evolutionarily conserved pathway. In the canonical processing pathway, this longer primary transcript, called the pri-miRNA, forms a characteristic hairpin structure that is recognized by the microprocessor complex (consisting of Drosha and DGCR8), cleaved into a pre-miRNA ~60 nt in length, and exported to the cytoplasm via an Exportin 5 and Ran-GTP complex. The ends of the pre-miRNA are then cleaved by Dicer to form a miRNA duplex, which consists of 5′ phosphates and 2 nt overhangs on each 3′ end. One strand of the miRNA duplex, the guide strand, is loaded onto an Argonaute protein and selected to form the RNA-induced silencing complex (RISC) containing the mature 22 nt miRNA (see recent reviews by Anastasiadou et al., 2018a; Bartel, 2018). The mature miRNA functions by binding to the 3′ untranslated regions (3′UTR) of mRNAs and inhibiting their use by either degradation or translational repression (Bartel, 2018; Esquela-Kerscher and Slack, 2006). Multiple studies have attempted to annotate the number of miRNAs in different species. For humans, higher estimates from miRBase v22 have placed the number of mature miRNAs at 2,654, but algorithms from other databases, such as MirGeneDB2.0, decrease this number to 588 high confidence miRNAs (Fromm et al., 2015; Kozomara et al., 2019). Despite discrepancies in their absolute numbers, it is evident that miRNAs have far-reaching effects on downstream processes, as more than 60% of coding genes are potential targets of miRNAs (Friedman et al., 2009). In addition, hundreds of miRNAs are conserved at their seed region across phylogeny (Bartel, 2018), suggesting key roles in developmental or physiological processes in animals.

Regarding cancer, miRNAs provide a powerful new avenue for the discovery of novel genetic risk factors (Ryan et al., 2010). Among the small ncRNA species, miRNAs are by far the most extensively studied in cancer compared to tsRNAs and piRNAs. miRNAs have been found to be altered in all cancer types studied (Volinia et al., 2006), and alterations in miRNAs have been demonstrated to play a crucial role in affecting molecular and cellular processes of the cancer state (Esquela-Kerscher and Slack, 2006; Nicoloso et al., 2009). Although researchers are still learning the extent of the contribution of miRNAs to cancer, these small ncRNAs seem to function in one of two ways—as tumor suppressors or oncogenes (commonly referred to as oncomiRs) that promote cancer growth or metastasis (Rupaimoole and Slack, 2017). Although small, miRNAs are powerful, with each molecule often able to regulate more than one target, and, vice versa, mRNAs are frequently targeted by several miRNAs (Bartel, 2018). As such, miRNAs function as master regulators that control the expression of thousands of coding and

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DOMANDE ARGOMENTO SPECIFICO

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1) Ruolo dei ncRNA come biomarcatori nei tumori
2) I microRNA come regolatori dell'espressione genica nei tumori - DOMANDA ESTRATTA