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Review

Developmental control of imprinted expression by macro non-coding RNAs

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ABSTRACT

Genomic imprinting is a developmentally regulated epigenetic phenomenon. The majority of imprinted genes only show parent-of-origin specific expression in a subset of tissues or at defined developmental stages. In some cases, imprinted expression is controlled by an imprinted macro non-coding RNA (ncRNA) whose expression pattern and repressive activity does not necessarily correlate with that of the genes whose imprinted expression it controls. This suggests that developmentally regulated factors other than the macro ncRNA are involved in establishing or maintaining imprinted expression. Here, we review how macro ncRNAs control imprinted expression during development and differentiation and consider how this impacts on target choice in epigenetic therapy.

Contents

1. Introduction

Mammals are diploid organisms and while the majority of genes are equally expressed from both chromosomes, a subset are subject to genomic imprinting and show maternal- or paternal-specific expression [1]. To date, 143 imprinted mouse genes are known that are mostly grouped into clusters [2]. Imprinted expression within a cluster is controlled by epigenetic mechanisms that act *in cis* (i.e.: on the same chromosome). The master regulator controlling expression of all genes in a cluster is the imprint control element (ICE) [3]. Each ICE is epigenetically marked on either the maternal or the paternal allele by a DNA methylation "imprint" acquired during gametogenesis and maintained on the same parental chromosome in diploid cells of the embryo after fertilization. The six best-studied mouse imprinted gene clusters in which the ICE was identified by deletion experiments are the *Igf2r*, *Kcnq1*, *Igf2*, *Gnas*, *Dlk1* and *Pws*/*As* clusters (reviewed in [4]).

All defined ICEs control expression of a macro or long non-protein-coding RNA (ncRNA) (defined here as a ncRNA >200 bp that is not processed to smaller RNAs). However, allele-specific silencing occurs by different mechanisms. For example, imprinted expression at the *Igf2* cluster arises because the ICE acts as a methylation-sensitive insulator interacting with CTCF and cohesin only on the unmethylated maternal allele that exclusively expresses the *H19* ncRNA (reviewed in [4]). Imprinted expression at the *Igf2r* and *Kcnq1* clusters (Fig. 1) is controlled, respectively, by the *Airn* and *Kcnq1ot1* macro ncRNAs, whose methylation-sensitive promoter lies in the ICE [5,6]. *Airn* and *Kcnq1ot1* are exclusively transcribed from the paternal allele as the gametic DNA methylation imprint represses the maternal promoter. Truncation experiments in mice that shortened these ncRNAs to 5% of their length show that they control paternal silencing of all genes in their cluster in embryonic and placental tissues [7,8].

Macro ncRNAs are now known to be widespread in the mammalian genome and are thought to function as transcriptional regulators although few have been studied in detail [9]. Here, we review the developmental regulation of imprinted macro ncRNAs, focusing on the *Airn* and *Kcnq1ot1* ncRNAs that play a functional silencing role. We consider how ncRNA developmental regulation

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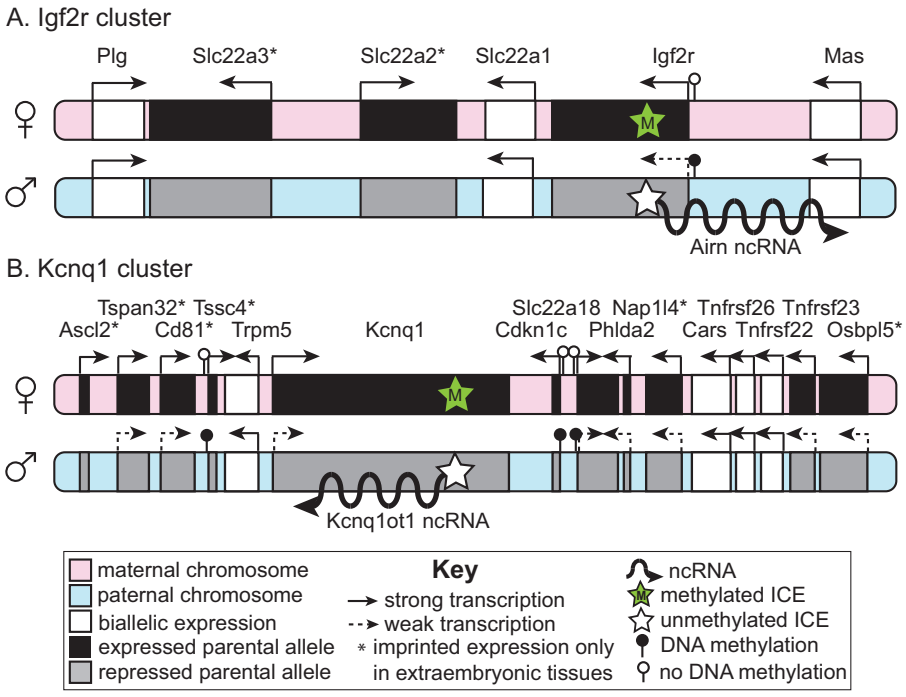

Fig. 1. Two mouse imprinted gene clusters containing a regulatory macro ncRNA. Genomic organization of the mouse *Igf2r* (A) and *Kcnq1* (B) imprinted gene clusters.

impacts on imprinted expression of flanking protein-coding genes. Finally, we discuss the choice of macro ncRNAs as targets in epigenetic therapy.

2. Imprinted macro ncRNAs are developmentally regulated

Genome-wide studies of macro ncRNAs revealed developmental and tissue-specific expression patterns, suggesting they are not unspecific "transcriptional noise" [9]. The expression of imprinted macro ncRNAs is also highly regulated (Table 1), and in most cases, imprinted expression of flanking protein-coding genes in the cluster correlates with ncRNA expression. The paternally expressed *Kcnq1ot1* macro ncRNA displays the most widespread expression pattern: it is found already in preimplantation embryos from the two-cell stage and maintained throughout mouse development, correlating with imprinted expression of flanking protein-coding genes [10,11]. Production of the *Airn* macro ncRNA correlates with *Igf2r* imprinted expression. *Airn* is absent from post-mitotic

Table 1
Developmental- and tissue-specific regulation of imprinted macro ncRNAs.

Imprinted cluster	ncRNA	Developmental-specific expression	Tissue-specific expression
Igf2r	*Airn*	From implantation stage; differentiated ESCs [24]	Ubiquitous, except neurons [12]
Kcnq1	*Kcnq1ot1*	From 2-cell stage [10]	Ubiquitous [11]
Igf2	*H19*	From blastocyst stage; differentiated ESCs [53]	Embryonic and extraembryonic tissues [15,53]; adult: skeletal muscle, thymus, heart, lung [53]
	Igf2as	From E10.5 till postnatal day 4 [54]	Skull, muscle, placenta [54]
Gnas	*Nespas*	E10.5 and E15.5[a]; adult [17–19]	Widespread [17–19]
	Exon1A	n.a.	Ubiquitous [55]
Dlk1	*Gtl2*	Continuous [56]	Widespread in fetus [21]; adult: brain, testis, spinal cord, skeletal muscle [57]
	Rtl1as	From E8.5[a] [58]	Brain [57]
	Rian	From E9.5 [57]	Extraembryonic tissues; embryo: brain, somites and cartilage; adult: brain, testis, skin, heart, muscle [57]
	Mirg	From E11.5 [57]	Embryonic and extraembryonic tissues [59]; adult: mainly brain, limbs, tongue, skin, testis [57]
	Mico1	From E8.5[a] [60]	Embryo: brain, heart, branchial arches; adult: mainly brain, pituitary, spleen, kidney, uterus [60]
	Mico1os	From E8.5[a] [60]	Embryo: brain, heart, branchial arches; adult: mainly brain, pituitary, spleen, kidney, uterus [60]
Pws/As	*Lncat*[b]	From E10.5 [16]	Post-mitotic neurons [16]

Abbreviations: n.a.: not analyzed; ESCs: embryonic stem cells; E: embryonic day; ubiquitous: expressed in all tested tissues.
[a] Not tested earlier.
[b] Includes U exons, *Ube3a-ats*, *Ipw* and *Pwcr1* ncRNAs.