Small nucleolar RNAs are concomitantly down-regulated during murine gamma delta T-cell development.

Shahan Mamoor¹

¹Thomas Jefferson School of Law

San Diego, CA 92101

mamoorsk@tjssl.edu

Gamma delta T-cells are a lymphocyte subset that display gamma delta T-cell receptors rather than the alpha beta T-cell receptors that alpha beta T cells like CD4 helper and CD8 cytotoxic T-cells display, and whose function straddles the intersection of innate and adaptive immune cells (1). To understand the transcriptional behavior of gamma delta T-cells during mammalian development, we performed global comparative differential gene expression profiling of datasets encompassing transcriptome data from embryonic and adult gamma delta T-cells from mice (2). These analyses revealed a species of non-coding RNA termed small nucleolar RNA, or snoRNA were among the most differentially expressed genes when comparing embryonic and adult gamma delta T-cells. Moreover, these snoRNA were uniformly down-regulated over the course of gamma delta T-cell development. These data demonstrate unprecedented developmental repression of snoRNA in lymphocytes and suggest that stage-specific repression of snoRNAs may serve some vital developmental purpose in the function of gamma delta T-cells.

Keywords: gamma delta T-cells, snoRNA, non-coding RNA, lymphocyte development, $\gamma\delta$ T-cell, $T\gamma\delta$.

Introduction

Gamma delta T-cells are a type of T-lymphocyte that are defined by their usage of gamma and delta chains of the Tcr gene locus at the T-cell receptor (3,4). They inhabit diverse anatomical sites such as the skin, the blood, thymus, tonsils and the spleen (5), and perform diverse functions from specific recognition of organic ligands (6) to parasite defense (7). The developmental origin of gamma delta T-cells is considered identical to that of alpha beta T-cells until sometime during the double negative 2 (DN2) or DN3 thymocyte maturation stages wherein beta selection, the process by which thymocytes express the beta chain of the TCR and choose the alpha beta lineage, at which point the lineage of the alpha beta T-cell diverges from that of the gamma delta T-cell (8,9,10,11,12,13,14).

To define the most significant transcriptional changes in the developmental program of gamma delta T-cells, we performed comparative gene expression profiling of mammalian gamma delta T-cells from embryonic day 18 and 6 weeks of age from mice using a published dataset (2). This analysis revealed that a class of non-coding RNA or ncRNA termed small nucleolar RNAs, or snoRNAs are differentially expressed in a stage-specific fashion and that these snoRNA transcripts uniformly decrease in expression through development.

Methods

GSE71637 (2) was utilized for this differential gene expression analysis, performed using the GEO2R web application, which identifies the most differentially expressed genes in any given comparison. Muñoz-Ruiz et al. used Affymetrix Mouse Gene 2.1 ST Array to perform global transcriptional profiling in wild-type and CD3H deficient mice; these data were extracted from this dataset. For all analyses on GEO2R, p-value adjustment was set to “Benjamini and Hochberg”, log-transformation of data was set to “auto-detect”, and the “NCBI generated” category of platform annotation was selected. Statistical analysis was performed with PRISM 8.1.2 (227) using an unpaired t-test with Welch’s correction.

Results

snoRNA are among the most differentially expressed genes when comparing gamma delta T-cells across development.

We used GEO2R to perform comparative global differential gene expression analysis, to identify the genes whose expression is most different between day 18 embryonic gamma delta T-cells and adult gamma delta T-cells at 6 weeks of age. Twenty-seven of the top five hundred most differentially expressed genes were ncRNA species including anti-sense RNA transcripts, pseudogenes, small nuclear RNA (snRNA), and microRNA (miRNA) (2) (S. Mamoor, analysis unpublished). Small nucleolar RNA, or snoRNA represented twelve of these ncRNA (Table 1). These snoRNA included snord16a, snord82, and snord87 as well as nine other snoRNA transcripts (Table 1 and Figure 2).

Of the most differentially expressed non-coding RNA, snoRNA but not miRNA are uniformly down-regulated during gamma delta T-cell development.

Analysis of the most differentially expressed ncRNA transcripts across murine gamma delta T-cell development revealed that while snoRNA and miRNA transcripts revealed that while the expression of these differentially expressed miRNA both increased and decreased through development (Figure 1B and Figure 3), the expression of each of the twelve most differentially expressed snoRNAs strictly decreased over development (Figure 1A and Figure 2).

Discussion

The central dogma of molecular biology (15) is that genes encoded by DNA are transcribed by transcriptional machinery (e.g., RNA polymerases) in a process known as transcription, and the resultant RNA templates are, in a process known as translation, translated into protein by machinery including the signal recognition particle, smooth and rough ribosomes and tRNA. It has since become apparent that some RNAs do not translate into

proteins; these transcripts are known as non-coding RNA (16). Though the function of many non-coding RNA remains elusive, the function of some has been elucidated. Xist is a non-coding RNA that functions in the maternal imprinting process (17), wherein one copy of the X chromosome is silenced by coating of that chromosome with Xist non-coding RNA transcripts, which recruits further epigenetic machinery like the polycomb repressive complex PRC2 (18,19), which methylates lysine 27 of Histone H3 (H3K27) on chromosomal DNA in order to enforce heterochromatin formation (compaction into dense, inactive DNA). Another non-coding RNA, Hotair similarly recruits PRC2, as well as the H3K4 lysine specific demethylase LSD1 to the HoxD locus (20,21), and becomes significantly active in cancers of the pancreas, breast, and stomach (22,23,24,25).

The nucleolus is a sub-organelle within the nucleus where specific transcription of ribosomal RNA, including 18S and 28S rRNA occurs (26). The nucleolus has specific targeting signals associated with transport and delivery of molecules therein, including PLLKKIKQ for c-myc, PQQKKKP for p53, GRKKRRQRRRAP for the HIV-1 Tat protein (27) and RRRGL for angiogenin (28). SnoRNA are a class of non-coding RNA that are associated with the nucleolus (29).

SnoRNAs can be generally be classified into two main groups, known as C/D box and H/ACA box snoRNAs (29). snoRNAs have been described to facilitate site-specific pseudouridylation and 2'-O-methylation of rRNAs (30). C/D box snoRNAs function as guiding templates for 2'-O-methylation of rRNA, while H/ACA box snoRNAs function likewise for the pseudouridylation of rRNAs (31,32). Though one study has identified a species of non-coding RNA known as sno-derived RNA, or sdRNA as markers of cancer immunity (33), there is no known role for snoRNA in the function or development of gamma delta T-cells, nor has it been reported that snoRNA are differentially expressed in gamma delta T-cells. In mouse macrophages, a long intergenic non-coding RNA lincRNA-Cox2 has been described to function in the activation and repression of immune genes *in vitro* and *in vivo* (34, 35), but a

similar role or function has not yet been defined for snoRNA in lymphocytes, in general, nor could we identify any literature describing a role for any type of ncRNA in gamma delta T-cells.

These results demonstrate not only that snoRNA are among the most differentially expressed genes in murine gamma delta T-cell development, but that the expression of these differentially expressed snoRNA uniformly decreases over time; in this study, from embryonic day 18 to 6 weeks of age. While miRNA were also differentially expressed (seven in total), the expression of these differentially expressed miRNA both increased and decreased over gamma delta T-cell development. Twelve separate snoRNA were identified in this global differential gene expression analysis of murine gamma delta T-cells. The function of these snoRNA in gamma delta T-cells is unknown. It is possible that site-specific pseudouridylation or 2'-O-methylation of rRNA or some other substrate RNA message may be essential for gamma delta T-cell development, or that repression of site-specific pseudouridylation or 2'-O-methylation of rRNA or some other substrate RNA message may be required for the developmental transition from embryonic to adult gamma T-cell.

To our knowledge, these are the first data to demonstrate differential and stage-selective expression of snoRNA during gamma delta T-cell development. These results merit further investigation into the role of snoRNA during lymphocyte development, specifically of the gamma delta T-cell.

References

1. Born, Willi K., Christopher L. Reardon, and Rebecca L. O'Brien. "The function of $\gamma\delta$ T cells in innate immunity." *Current opinion in immunology* 18.1 (2006): 31-38.
2. Muñoz-Ruiz, Miguel, et al. "TCR signal strength controls thymic differentiation of discrete proinflammatory $\gamma\delta$ T cell subsets." *Nature immunology* 17.6 (2016): 721.
3. Ishida, Isao, et al. "T-cell receptor gamma delta and gamma transgenic mice suggest a role of a gamma gene silencer in the generation of alpha beta T cells." *Proceedings of the National Academy of Sciences* 87.8 (1990): 3067-3071.
4. Haas, Werner, Pablo Pereira, and Susumu Tonegawa. "Gamma/delta cells." *Annual review of immunology* 11.1 (1993): 637-685.
5. Falini, B., et al. "Distribution of T cells bearing different forms of the T cell receptor gamma/delta in normal and pathological human tissues." *The Journal of Immunology* 143.8 (1989): 2480-2488.
6. Belmant, Christian, et al. "A chemical basis for selective recognition of nonpeptide antigens by human δ T cells." *The FASEB Journal* 14.12 (2000): 1669-1670.
7. Elloso, M. Merle, et al. "Inhibition of Plasmodium falciparum in vitro by human gamma delta T cells." *The Journal of Immunology* 153.3 (1994): 1187-1194.
8. Burtrum, Douglas B., et al. "TCR gene recombination and alpha beta-gamma delta lineage divergence: productive TCR-beta rearrangement is neither exclusive nor preclusive of gamma delta cell development." *The Journal of Immunology* 157.10 (1996): 4293-4296.
9. Ciofani, Maria, et al. "Stage-specific and differential notch dependency at the $\alpha\beta$ and $\gamma\delta$ T lineage bifurcation." *Immunity* 25.1 (2006): 105-116.
10. Aifantis, Iannis, et al. "On the Role of the Pre-T Cell Receptor in $\alpha\beta$ versus $\gamma\delta$ T Lineage Commitment." *Immunity* 9.5 (1998): 649-655.
11. Tanigaki, Kenji, et al. "Regulation of $\alpha\beta/\gamma\delta$ T cell lineage commitment and peripheral T cell responses by Notch/RBP-J signaling." *Immunity* 20.5 (2004): 611-622.
12. Kreslavsky, Taras, et al. "T cell receptor-instructed $\alpha\beta$ versus $\gamma\delta$ lineage commitment revealed by single-cell analysis." *Journal of Experimental Medicine* 205.5 (2008): 1173-1186.
13. Garbe, Annette I., et al. "Differential synergy of Notch and T cell receptor signaling determines $\alpha\beta$ versus $\gamma\delta$ lineage fate." *Journal of Experimental Medicine* 203.6 (2006): 1579-1590.
14. Ciofani, Maria, and Juan Carlos Zúñiga-Pflücker. "Determining $\gamma\delta$ versus $\alpha\beta$ T cell development." *Nature Reviews Immunology* 10.9 (2010): 657.
15. Crick, Francis. "Central dogma of molecular biology." *Nature* 227.5258 (1970): 561.
16. Mattick, John S., and Igor V. Makunin. "Non-coding RNA." *Human molecular genetics* 15.suppl_1 (2006): R17-R29.
17. Penny, Graeme D., et al. "Requirement for Xist in X chromosome inactivation." *Nature* 379.6561 (1996): 131.
18. Sarma, Kavitha, et al. "ATRX directs binding of PRC2 to Xist RNA and Polycomb targets." *Cell* 159.4 (2014): 869-883.

19. da Rocha, Simão Teixeira, et al. "Jarid2 is implicated in the initial Xist-induced targeting of PRC2 to the inactive X chromosome." *Molecular cell* 53.2 (2014): 301-316.
20. Wu, Liang, et al. "Binding interactions between long noncoding RNA HOTAIR and PRC2 proteins." *Biochemistry* 52.52 (2013): 9519-9527.
21. Li, Lingjie, et al. "Targeted disruption of Hotaair leads to homeotic transformation and gene derepression." *Cell reports* 5.1 (2013): 3-12.
22. Kim, Kyoung Hyun, et al. "HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer." *Oncogene* 32.13 (2013): 1616.
23. Gupta, Rajnish A., et al. "Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis." *Nature* 464.7291 (2010): 1071.
24. Sørensen, Kristina P., et al. "Long non-coding RNA HOTAIR is an independent prognostic marker of metastasis in estrogen receptor-positive primary breast cancer." *Breast cancer research and treatment* 142.3 (2013): 529-536.
25. Liu, Xiang-hua, et al. "Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer." *Molecular cancer* 13.1 (2014): 92.
26. Boisvert, François-Michel, et al. "The multifunctional nucleolus." *Nature reviews Molecular cell biology* 8.7 (2007): 574.
27. Dang, Ch V., and W. M. Lee. "Nuclear and nucleolar targeting sequences of c-erb-A, c-myc, N-myc, p53, HSP70, and HIV tat proteins." *Journal of Biological Chemistry* 264.30 (1989): 18019-18023.
28. Moroianu, Junona, and James F. Riordan. "Identification of the nucleolar targeting signal of human angiogenin." *Biochemical and biophysical research communications* 203.3 (1994): 1765-1772.
29. Samarsky, Dmitry A., et al. "The snoRNA box C/D motif directs nucleolar targeting and also couples snoRNA synthesis and localization." *The EMBO Journal* 17.13 (1998): 3747-3757.
30. Kiss, Tamás. "Small nucleolar RNA-guided post-transcriptional modification of cellular RNAs." *The EMBO journal* 20.14 (2001): 3617-3622.
31. Ni, Jingwei, Amy L. Tien, and Maurille J. Fournier. "Small nucleolar RNAs direct site-specific synthesis of pseudouridine in ribosomal RNA." *Cell* 89.4 (1997): 565-573.
32. Kiss-László, Zsuzsanna, et al. "Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs." *Cell* 85.7 (1996): 1077-1088.
33. Chow, Ryan D., and Sidi Chen. "Sno-derived RNAs are prevalent molecular markers of cancer immunity." *Oncogene* 37.50 (2018): 6442.
34. Atianand, Maninjay K., and Katherine A. Fitzgerald. "Long non-coding RNAs and control of gene expression in the immune system." *Trends in molecular medicine* 20.11 (2014): 623-631.
35. Carpenter, Susan, et al. "A long noncoding RNA mediates both activation and repression of immune response genes." *science* 341.6147 (2013): 789-792.

Table 1

Species	Rank	p-value	Chromosome	ID
ENSMUST00000157287 // ncRNA:snoRNA	20	0.00004042	chr14	17304512
ENSMUST00000104252 // ncRNA:snoRNA	43	0.00006299	chr10	17239755
ENSMUST00000157820 // ncRNA snoRNA	53	0.00007607	chrX	17543194
small nucleolar RNA C/D box 87 Snord87	91	0.00011523	chr1	17221191
ENSMUST00000083425 // ncRNA:snoRNA	118	0.00014807	chr9	17518316
ENSMUST00000158284 //ncRNA:snoRNA	126	0.0001615	chr19	17356425
Snord16a	129	0.00016562	chr9	17518312
ENSMUST00000082608 // ncRNA:snoRNA	155	0.00019557	chr4	17428795
ENSMUST00000104449 // ncRNA:snoRNA	157	0.00019969	chr11	17252847
ENSMUST00000082738 // ncRNA:snoRNA	228	0.00029172	chr13	17287336
ENSMUST00000116972 // ncRNA:snoRNA	265	0.00036898	chr2	17374316
Snord82	289	0.00041772	chr1	17225173

Table 1: snoRNA are among the most differentially expressed genes when comparing murine gamma delta T-cells across development.

The rank of each snoRNA transcript based on statistical significance of global differential expression, the corresponding p-value, chromosomal location of the gene encoding that transcript, and transcript ID provided by NCBI GEO2R are shown.

Figure 1: snoRNA but not miRNA are differentially expressed and down-regulated in a developmental stage-specific manner in murine gamma delta T-cells.

Figure 1A: The RNA expression value of each differentially expressed snoRNA transcript, in wild-type and CD3H deficient mice ($n=2$ for developmental stage; each n of 2 includes 1 sample each from day 18 embryos and adult mice 6 weeks of age) is displayed.

Figure 1: snoRNA but not miRNA are differentially expressed and down-regulated in a developmental stage-specific manner.

Figure 1B: The RNA expression value of each differentially expressed miRNA transcript, in wild-type and CD3H deficient mice ($n=2$ for developmental stage; each n of 2 includes 1 sample from day 18 embryos and adult mice 6 weeks of age) is displayed.

Figure 2: snoRNA are differentially expressed and down-regulated in a developmental stage-specific manner in murine gamma delta T-cells.

The RNA expression value of each differentially expressed snoRNA transcript, in wild-type and CD3H deficient mice ($n=2$ for developmental stage; each n of 2 includes 1 sample from day 18 embryos and adult mice 6 weeks of age) is displayed. The error bars represent the standard deviation of the mean, and the p-value is denoted on each graph. Though the p-value may be greater than .05 in some cases, these p-values are comparing the significance of the difference between the actual RNA expression values, and not the statistical significance of global differential expression as determined by GEO2R, shown in Table 1.

Figure 3: miRNA are differentially expressed in a developmental stage-specific manner in murine gamma delta T-cells.

The RNA expression value of each differentially expressed miRNA transcript, in wild-type and CD3H deficient mice ($n=2$ for developmental stage; each n of 2 includes 1 sample from day 18 embryos and adult mice 6 weeks of age) is displayed. The error bars represent the standard deviation of the mean, and the p-value is denoted on each graph. Though the p-value may be greater than .05 in some cases, these p-values are comparing the significance of the difference between the actual RNA expression values, and not the statistical significance of global differential expression as determined by GEO2R, shown in Table 1.