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ABSTRACT

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biology [9]. In pancreatic cancer, miR-29a has been demonstrated to be down-regulated in pancreatic cancer cell-lines, and over-expression of miR-29a decreases proliferation, leading miR-29a to be labelled a tumour-suppressor-miR [10]. However, several studies suggest that the in vivo expression does not correlate with the in vitro results, as miR-29 is upregulated in pancreatic cancer surgical specimens [11, 12], indicating it as a potential oncomir. In other contexts, miR-29a has been demonstrated to be an oncomir. In leukemia, miR-29a is upregulated in indolent human B cell chronic lymphocytic leukemia and acute myeloid leukemia, and spontaneous leukemia forms in mice which over-express miR-29a in B cells or myeloid cells [13–15].

With conflicting expression data on miR-29a in pancreatic cancer, and a demonstrated oncogenic function in other cancer types, it is important to direct test the function of miR-29a in in vivo murine models. Here we used miR-29a knockout mice and the TAg transgenic model of pancreatic acinar carcinoma to investigate the functional role of miR-29a in a mouse model of pancreatic cancer, using the acinar subtype. We found no functional role for miR-29a in the onset or growth of pancreatic acinar carcinoma, or in the death rate of tumour-bearing mice, indicating that miR-29a is oncogenically neutral.

RESULTS

In order to directly test the in vivo function of miR-29a in pancreatic acinar carcinoma, we intercrossed the miR-29a-deficient mouse strain (deficient in both miR-29a and miR-29b-1) [16] with the spontaneous pancreatic acinar carcinoma Ela1-TAg transgenic strain described above [3]. TAg mice, wildtype, heterozygous or knockout for miR-29a, were monitored for pancreatic acinar carcinoma development through MRI assessment every two weeks, from the age of 7 weeks. MRI assessment allowed the detection of tumours (Figure 1A). For both female (Figure 1B) and male (Figure 1C) mice, no significant difference was observed in the cumulative incidence of pancreatic acinar carcinoma. When assessing the age of first tumour detection, no significant effect of miR-29a genotype was observed for male mice (Figure 1D), with only a minor impact of delayed tumour onset observed for female mice (Figure 1D).

To determine the impact of miR-29a on pancreatic acinar carcinoma growth post-development, miR-29a wildtype, heterozygous and knockout mice were longitudinally monitored from first cancer detection to death, excessive morbidity or 21 weeks of age. MRI assessment allowed longitudinal tumour growth tracking. Within each individual mouse the total number of tumours and cross-sectional maximal size was measured, allowing the calculation of total tumour volume. Despite the variation in first tumour detection, post-detection each tumour grew in a classical exponential growth fashion, regardless of sex or miR-29a genotype (Supplementary Figure 1, Figure 2A). A linear mixed-effect model found no significant differences in tumour curves. In order to directly compare the growth rates of tumours within each mouse, we square root transformed total tumour volume and plotted tumour growth from time of first detection (Supplementary Figure 2, Figure 2B). Direct comparison of tumour growth rates was performed as the percentage of tumour volume increase between MRI measurement (every 14 days, averaged per mouse over the entire course of tumour measurement). For both male and female mice, no change in the tumour growth rate was observed in miR-29a heterozygous or miR-29a knockout mice (Figure 2C). As an independent approach, we assessed tumours from wildtype and knockout mice by histology and immunofluorescence, observing no consistent differences

Prior to this study, miR-29a was an attractive potential therapeutic target in pancreatic cancer. miR-29a is known to maintain self-renewal capacity in haematopoietic stem cells [20], a property which leads to oncogenic potential when over-expressed in the B cell progeny [13, 14]. Targets of miR-29a include key pro-survival genes (Bcl2 and Mcl1) [21], and elevated miR-29c expression (with the same regulation-inducing seed

Figure 1: No effect of miR-29a on tumour onset in a pancreatic cancer model. Ela1-TAg$^+$ mice, on the wildtype, miR-29a heterozygous and miR-29a knockout backgrounds, were monitored for pancreatic cancer detection by MRI every two weeks. (A) Representative MRI scans for wildtype (top) and miR-29a knockout (bottom) mice, at 9 weeks, 15 weeks and 21 weeks. Arrows indicate detected tumours. (B) Cumulative incidence of pancreatic cancer as a function of age at tumour onset in wild-type, heterozygous and miR-29a-deficient mice, for female and (n = 24, 11, 9) (C) male (n = 21, 14, 10). The P values were calculated using the log-rank test. (D) Violin plots showing the mean, standard deviation and kernel probability density of the age at tumour onset under each condition in female (upper panel) and male (lower panel) mice. The P values were calculated using two-sided Mann-Whitney U-test.

Figure 2: No effect of miR-29a on tumour growth in a pancreatic cancer model. Ela1-TAg$^+$ mice, on the wildtype, miR-29a heterozygous and miR-29a knockout backgrounds, were monitored for pancreatic cancer growth by MRI every two weeks. (A) Total tumour volume was estimated at each time-point for wildtype, heterozygous and knockout female mice, and wildtype, heterozygous and knockout male mice (n = 24, 11, 9, 21, 14, 10). Each line indicates average tumour size across the group. (B) Individual square root transformed total predicted tumour volume curves for wildtype, heterozygous and knockout female and male mice (n = 24, 11, 9, 21, 14, 10). Time 0 corresponds to the first detected tumour time-point and each line indicates tumour size in a single mouse. (C) Violin plots showing the mean, standard deviation and kernel probability density of the % tumour increase every two weeks under each condition in female and male mice (n = 24, 11, 9, 21, 14, 10). The P values were calculated using two-sided Mann-Whitney U-test.

sequence as miR-29a) is associated with resistance to treatment [22]. The

using the lmer function within lme4 (Linear Mixed-Effects Models using 'Eigen' and S4) package in R.

We computed estimates of a survival curve for censored data using the Kaplan-Meier estimator [29] that makes no parametric assumptions about the form of distribution (R package "npsurv"). Cumulative incidence curves were generated using the R package "survplot" with the fun=function(x) {1 - x} argument [30]. The comparison of cumulative incidence and survival distributions between two samples was performed using log-rank test implemented in the R "survdiff" package [31]. The logrank test is a non-parametric test and the most widely used method for comparing two or more survival curves [32]. Two group comparisons presented in the violin plots were