MiR-140-3p Downregulation in Association with PDL-1 Overexpression in Many Cancers: A Review from the Literature Using Predictive Bioinformatics Tools

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Abstract Programmed death-ligand 1 (PD-L1) has been speculated to play a critical role in suppression of the immune system and it can be upregulated in cancer cells, which may allow cancers to evade the host immune system. MicroRNAs (miRNAs) are small non-coding RNA molecules (containing about 22 nucleotides), that function in RNA silencing and post-transcriptional regulation of gene expression. MiRNAs were found deregulated (upregulated or downregulated) and implicated in cancer development with various roles which depend on their gene target. Using targetscan web server prediction algorithm, we concluded that miR-140-3p is a targeting mirRNA with conserved consequential pairing of target region for PD-L1. Moreover, by reviewing all the available cancer studies in Pub/Medline about miR-140-3p, was found permanently down regulated. Furthermore, in recent immunotherapy related clinical trials in most cancers, evaluated PD-L1, it is found overexpressed. In the near future, in vitro or in vivo studies need to validate whether there is direct correlation between PD-L1 overexpression and miR-140-3p downregulation as targetscan performed algorithm predicted.

Keywords Programmed cell death • Human cancer • miNA • miRNA • PD-L1

1 Introduction

PD-1 (Programmed cell death protein 1) is a key immune-checkpoint receptor expressed by activated T cells, which mediates immunosuppression. PD-1 functions primarily in peripheral tissues, where T cells may encounter the immunosuppressive PD-1 ligands, namely PD-L1 (B7-H1) and PD-L2 (B7-DC), which are expressed

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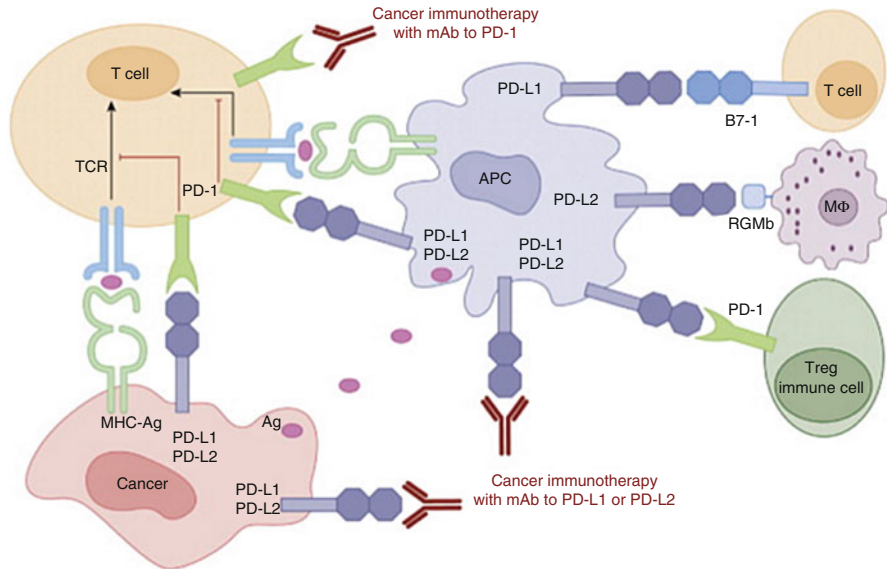
Fig. 1 Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway [5]

by tumor cells, stromal cells, or both [1]. It appears that upregulation of PD-L1 may allow cancers to evade the host's immune system through the transmission of an immunosuppressing signal from tumor cells. Many recent studies show that PD-L1 expression in tumor is associated with impaired survival [2]. An analysis of 196 tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased tumor aggressiveness and a 4.5-fold increased risk of death [3]. Therefore, many PD-L1 inhibitors are in development as immuno-oncology therapies, showing good results in clinical trials [4] and consist a new treatment approach against cancer treatment (Fig. 1).
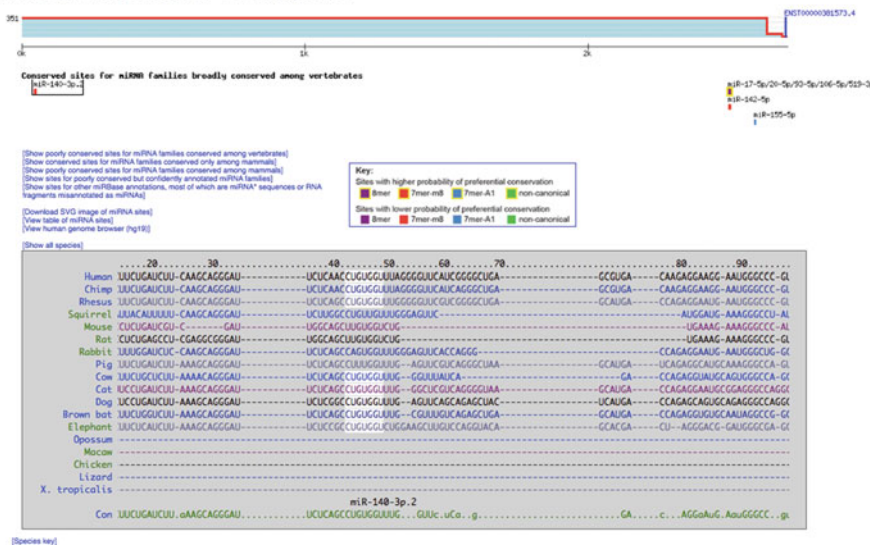
An effective strategy of preventing immunosuppression by antibodies blocking the PD-1, or its ligand (PD-L1), was shown in phase I trials by inducing a 30–50% response in several cancer types [6]. More recent phase II and III studies took accelerated approval of anti-PD-1 antibodies for metastatic melanoma (MM) [7, 8], non-small cell lung cancer (NSCLC) [9] and renal cell cancer (RCC) [10]. In another very recent phase II study atezolizumab, an anti-PD-L1 antibody was approved for metastatic bladder cancer treatment [11]. The importance of PD-L1 expression in prognosis and in prediction of treatment outcome, promote identifying other molecules modulators or regulators of it's expression. MicroRNAs (miRNAs) are a class of small, non-coding, endogenous single RNA molecules that play important roles in gene expression through binding to the 3 UTR of the targeted gene mRNA, leading to mRNA cleavage or translational repression [12]. miRNAs are transcribed by RNA polymerase II as part of capped and polyadenylated primary
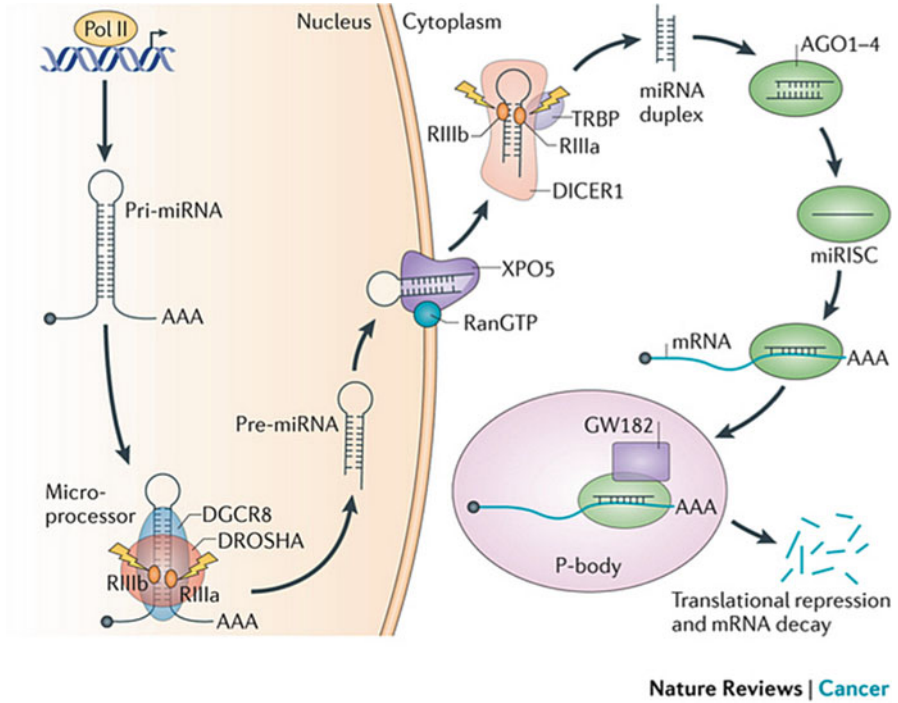
Fig. 2 Overview of miRNA biogenesis pathway [13]

transcripts (pri-miRNAs) that can be either protein-coding or non-coding. The primary transcript is cleaved by the Drosha ribonuclease III enzyme to produce an approximately 70-nt stem-loop precursor miRNA (pre-miRNA), which is further cleaved by the cytoplasmic Dicer ribonuclease to generate the mature miRNA and antisense miRNA star (miRNA*) products. The mature miRNA is incorporated into a RNA-induced silencing complex (RISC), which recognizes targeted mRNAs through imperfect base pairing with the miRNA and most commonly results in translational inhibition or destabilization of the target mRNA (Fig. 2).

Numerous studies show that miRNAs participate in various biological processes, such as cell differentiation, cell growth, apoptosis, death and timing development [14]. In the present study we aim to investigate if PD-L1 is a predicted target by microRnas, using the targetscan web server prediction algorithm. The results will be analyzed by reviewing the available publication studies of the target miRnas in various tumors and PD-L1 expression as well.

Human CD274 ENST00000381573.4   3' UTR length: 2705

Fig. 3 Results evince PD-L1 as predictive target for mir-140-3p

Fig. 4 mir-140-3p predicted conserved consequential pairing of 3-UTR mRNA for PD-L1

2 Method

In bioinformatics, TargetScan is a web server that predicts biological targets of microRNAs by searching for the presence of sites that match the seed region of each miRNA. Performing targetscan, results showed PD-L1 as predictive target for miR-140-3p (Fig. 3).

The prediction targetscan method indicates that miR 140-3p.2 predicted target PD-L1(CD274) with conserved consequential pairing of target region and miRna, in position 43–49, near the stop codon end of the 3-UTR, a region with high efficacy for repression [15] (Fig. 4).

   The prediction targetscan method, indicate that miR 140-3p.2 is the only miRna that predicted target PD-L1(CD274) with conserved consequential pairing of target region and miRna, in this position. Searching in Pubmed/Medline database, all available publications about: 'miR-140-3p and cancer', in almost all studies' results that referred to cells or tissues samples analysed, miR140-3p was found down regulated and higher levels of miR 140-3p expression, favored tumor's deprivation. In a study analyzing miR-140-3p in breast cancer cell lines, were found evidence that suggest there is functional synergy between the canonical hsa-miR-140-3p and the newly identified 5'isomiR-140-3p in suppressing growth and progression of breast cancer by simultaneously targeting genes related to differentiation, proliferation and migration. In patients with higher levels there was a trend for better survival [16]. In others studies, miR-140-3p expression level was lower in lung cancer tissues compared to adjacent normal lung cancer tissue and after miR-140-3p was upregulated in A549 or H1299 cells, cell proliferation, invasion and migration was notably attenuated [17, 18]. Moreover, analyzing miRNA expression signatures in angioimmunoblastic T-cell lymphomas (AITLs) of 760 microRNAs in 30 nodal AITL, miR-140-3p, was found to be downregulated to a significant level [19]. The same results found in multiple myeloma cells study, demonstrating miR-140-3p being repressed [20].

   In contrast, plasma levels of miR-140-3p were significantly higher in the papillary thyroid carcinoma (PTC) patients than in those with benign nodules or the healthy controls [21]. This is not supported by others studies referred to circulated miRnas in tumors. In a study assessing circulating microRna in blood in five different tumors, miR-140-3p was a potential biomarker, among those selected to distinguish patients with cancer or other diseases from healthy controls. Significantly miR-140-3p was downregulated in all five tumors samples (lung, ovarian, colon, nervous system, hematologic) [22]. Furthermore, from the rest of available studies evaluating miR-140-3p in cancer, published in pubmed database, the conclusion is the same. There was a clear miR-140-3p underexpression in all different tumor samples detected in these studies (cutaneous squamous cell carcinoma, basal cell carcinoma, colon adenocarcinoma, oral squamous cell carcinoma, ovarian carcinoma, lung squamous cell carcinoma, multiple myeloma) [23–29].

   Moreover, the expression of PD-l1 in various tumor types has been widely explored in context to predict the most effective immunotherapy for the disease. In studies evaluating PD-L1 expression, as a predictive biomarker in immunotherapy, solid tumors with most increased expression were in concordance with tumor types in which miR-140-3p was found downregulated before [30] (Fig. 5).

Tumor histology	Percent tumor samples expressing PD-L1	Tumor surface expression cutoff for positivity
Melanoma	38%-100%	>10%-5%
RCC	44% (primary tumor), 54% (metastasis)	10%-1%
NSCLC	49%-95%	11%-1%
Bladder	28%-21% (37% squamous, 22% transitional)	5%-1%
Head and neck	66%-31%	10%-5%
Ovarian	87%-87%	10%-5%
Cervical	29%-19%	εως 5%
Colorectal	56% [(MSI-H) vs. 21% (MSS)]-53%	>10%
Glioblastoma multiforme	45%-25%	50%-5%
Breast cancer	18% TNBC (0% ER/PR/HER2+)-50%	5%-1%
Esophageal	44%(squamos)-20%	10%-5%
Hepatocellular carcinoma	25%-15%	5%
Pancreatic	39%	10%
Gastric	42%	NR
Sarcoma	12% (27% GIST)	1%
Unknown primary	28%	5%
Acute myeloid leukemia	37%	5%
Leukemias (various)	57%	NR
B-cell lymphomas	58%	5%
Multiple myeloma	93%	NR

Fig. 5 Table 1

3 Discussion

The association of miR-140-3p with his target gene PD-L1, as was predicted by targetscan prediction algorithm, is more complex and unclear than it seems. In a study at chordomas, miR-140-3p was found upregulated and correlated with

recurrence, tumor invasion and worse recurrence-free survival [31]. However, at a recent study analyzing PD-L1 overexpression in tumor infiltrating lymphocytes, in spinal. In spinal chordoma samples, there was no association between PD-L1 with local recurrence-free survival (LRFS) or overall survival (OS), but instead with favorable prognosis [32]. Certainly, its apoptotic role is more elucidated in preclinical or clinical studies that used miR-140-3p, in fields other than in oncology. Preclinically, accessing miR-140-3p with others apoptotic miRnas in myocardial infarction post transplantation with skeletal myoblast in rats, there was apparently increased expression of this miRna, starting apoptosis [33]. Clinically, targeting synovial fibroblast by intraarticular delivery of miRNAs 140-3p and 140-5p can ameliorate autoimmune arthritis [34]. These findings indicate that miR-140-3p has an apparent role in apoptosis, which might implicate additionally PD-L1 regulation. All the above studies suggest that miR-140-3p functions through apoptosis network but the only way to assure that it is inversely correlated or even downregulate PD-L1 expression, are in vivo studies. In a previous study exploring miRNA profiling of normal mammary stem cells and cancer stem-like cells (CSC) from DCIS (ductal carcinoma in situ) tumors, revealed that miR-140 is significantly downregulated in cancer stem-like cells compared with normal stem cells [35]. Moreover, in ER-negative/basal-like DCIS, a cancer stem cell fenotype, restoration of miR-140-3p reduced tumor growth in vivo. Considering that PD-L1 in basal like breast cancer cells is significantly upregulated compare to others breast cancer cells [36] there is an obvious indirect correlation with miR-140-3p downregulation.

4 Conclusions

MiR140-3p, evaluated in many tissue cancers, has been found downregulated. Moreover, his target gene PD-L1, with high clinical importance in most cancer, is overexpressed. In view of the fact that, miR-140-3p is corroborated as apoptotic related miRna, and its ability in pairing conserved with his target PD-L1 in a unique way (near the stop codon end of the 3-UTR) as targetscan performed, makes warrant the confirmation with further in vivo or in vitro experiments as a direct PD-L1 regulator.

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