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GW182 associates with circulating microvesicles in human plasma

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Abstract

To date, microRNAs (miRNAs) in human plasma have been discovered within circulating microvesicles (cMV), bound to Argonaute 2 and associated with HDL and LDL microvesicles. The protein GW182 shares an association with both multivesicular bodies and the Argonaute family of proteins. GW182 has the capacity to bind all human Argonaute proteins (1-4) and their associated miRs. In the cell, GW182 is associated with the membrane of multivesicular bodies and has the ability to congregate Argonaute-loaded RISC complexes. In addition, GW182 has been observed on the surface of purified exosomes.

Here we investigated the relationship of GW182 with Argonaute and cMV in human plasma and urine. A monoclonal antibody directed toward GW182 was used to capture the protein. This isolate also contained Argonaute proteins as determined by Western analysis. The co-precipitation of GW182 and Argonaute suggests that these two proteins retain their functional relationship in plasma. RNA was then isolated from precipitates for miRNA detection and analysis. The GW182-associated miRNA profile from human plasma contained individual miRNAs whose abundance either equaled or surpassed that of their matched Argonaute 2 immuno-precipitated miRNAs. This implies that GW182 maintains an association with the family of Argonaute proteins and a subset of cMV in human plasma.

Methods

To prepare beads for the GW182 immunoprecipitation, 50 ul of Magnabind protein G beads (Thermo Scientific _Cat. # 21349) were placed in a 1.5 ml eppendorf tube and placed on a magnetic separator (New England Biolabs_Cat. # S1509S) for one minute. The storage buffer was removed and discarded. The beads were washed once with 200 ul of PBS. 2.5 ug of anti-GW182 antibody was allowed to bind the beads in 200 ml PBS for a period of 30 minutes at room temperature (RT.) The beads were washed three times with ice cold PBS. The beads were resuspended in 200 ul of PBS and mixed with 200 ul of normal human plasma. The mixture was allowed to roll O/N on a Thermo Scientific Labquake Shaker/Rotisserie at 4°C. The samples were washed in a mildly stringent buffer. Precipitates were analyzed by either Western or RNA was isolated and analyzed by RT-qPCR.

A plate-based ELISA was developed using 5 ug/ml GW182 capture and 2.5 ug/ml Ago2-biotin detection. Following plate coating the plates were blocked and plasma samples were captured overnight at 4 C. Wells were washed with PBS with 1% BSA. Streptavidin polyHRP was used at concentrations ranging from 1:20,000 to 1:40,000.

For urine, anti-GW182 was conjugated to Luminex beads and then blocked. The volume of urine sample tested was 25 ul. Pan Argonaute conjugated to PE was used for detection.

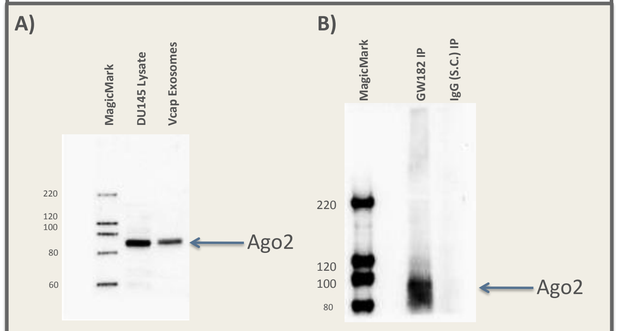
Results

The presence of Argonaute 2 was confirmed in purified VCaP exosomes by Western blot. Precipitation of GW182 from human plasma revealed an association with Ago2 by Western analysis. RNA was isolated from samples following IP from human plasma using either anti-Ago2 or anti-GW182. The copy number of known circulating miRNAs was comparable across the IPs.

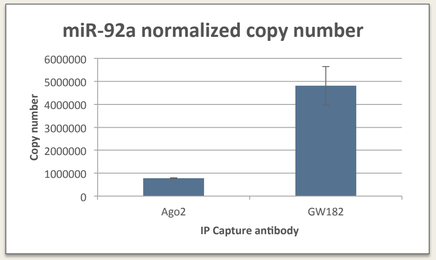
A plate-based ELISA was developed to evaluate the relationship of GW182 and Argonaute proteins in biological fluids. A signal that titrated with input was observed when GW182 was used as capture followed by Ago2 detection in either raw plasma or concentrated circulating microvesicles from plasma. Additional research sample were surveyed using the plate ELISA strategy. The levels of GW182:Ago2 positive particles varied dramatically across the sample set. Lastly, an association of GW182 and the Argonaute family of proteins was confirmed across five urine research samples using Luminex technology.

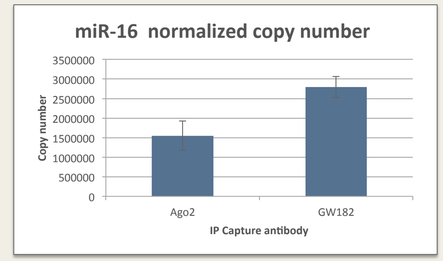
Conclusions

GW182 and Ago2 IP revealed strong IP of circulating RNA. Both miR-16 and miR-92a were enriched in AGO2 and GW182 IPs. This is the first observation of the use of GW182 for the purpose of surveying miRNAs from human plasma and urine. The potential source(s) of miRNA from human plasma and urine include microvesicles/exosomes and/or circulating Ago2-bound ribonucleoprotein complexes (RNP). This evidence suggests that GW182 may be a useful tool in the identification of a unique subpopulation of biomarkers in biological fluids.

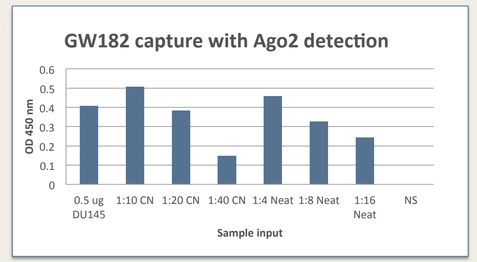


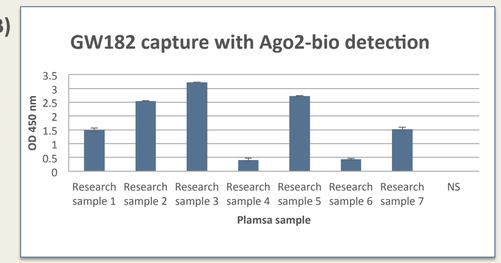
Ago2 co-precipitates with GW182 in human plasma. A) Western analysis for Ago2 in Du145 lysate and purified VCaP exosomes. **B)** IP of GW182 from human plasma reveals co-immunoprecipitation of Ago2 by Western blot.



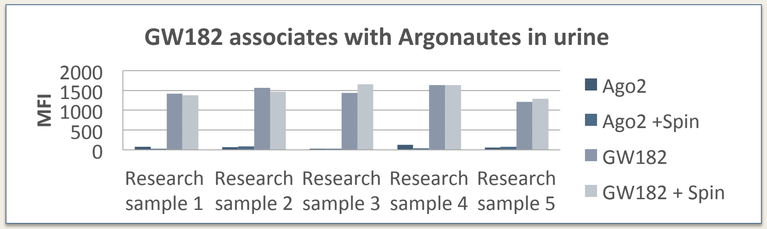


Immunoprecipitation of microRNA from human plasma. Anti-AGO2 (abcam, ab57113, lot GR29117-1), GW182 (Bethyl Labs, A302-330A) and IgG (Santa Cruz sc-2025) were conjugated to Magnabind protein G beads (Thermo Scientific _Cat. # 21349). Conjugated beads were incubated with human plasma. RNA was isolated and screened for select microRNAs (miR-16 and miR-92a) using ABI Taqman detection kits (ABI_391 and ABI_431), respectively. RNA was quantified against synthetic standards and normalized to IgG control.





Sandwich ELISA confirms association of GW182 with Ago2 in human plasma. A) Titration of sample input using purified microvesicles and raw plasma by plate-based ELISA using anti-GW182 as a capture (GW182 (Bethyl Labs, A302-330A) and biotinylated anti-Ago2 (abcam, ab57113, lot GR29117-1) as a detector. Signal normalized to no sample control. **B)** A survey of seven research samples reveals variable levels of GW182:Ago2 binding in human plasma. Signal normalized to no sample control.



GW182 associates with Argonautes in human urine. The relationship between human GW182 and the Argonaute family of proteins was investigated in urine using Luminex. GW182 capture followed by Pan Argonaute detection was tested across five research samples. Conditions included raw vs cell + hard spun urine.