Chimeric type I and type III and PS -targeting IFN fusion proteins for therapeutic applications

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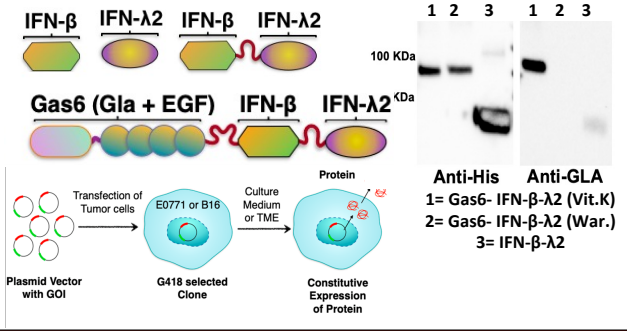
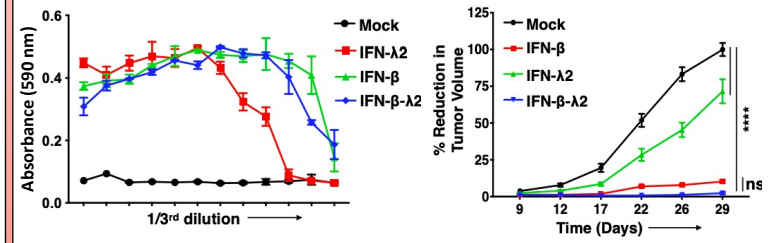
Background Type I and type III IFN receptors (IFNAR and IFNLR) are functionally compartmentalized, IFNAR is broadly expressed, but IFNLR is expressed on epithelial cells like cells lining the respiratory, gastrointestinal, and reproductive tracts. Here, in an attempt to enhance the action of IFNs at epithelial-immune cell interfaces, we have generated chimeric fusion proteins that fuse type I and type III IFNs into a single polypeptide chain separated by a flexible linker. Such fusion proteins retain functions of both type I and type III IFNs but show enhanced antiviral and anti-tumor activities compared to the combined action of their monomeric counterparts. Furthermore, we also fused the phosphatidylserine (PS)-binding Gla domain of Gas6 to the IFN proteins rendering them capable of preferentially localizing to PS rich target sites such as tumors and acutely stressed virus-infected tissues.

Methods IFN-β-λ2 fusion proteins were engineered as recombinant proteins with IFN-β and IFN-λ2 separated by a linker. Gas6-IFN-βλ-2 were engineered in a similar way using Gla+ EGF domains of Gas6 (Growth Arrest factor 6). Plasmids were stably transfected into EO771 cell lines that constitutively produce these fusion molecules. Transient transfection into HEK293T cells produced conditioned media containing fusion molecules which were used for in-vitro studies. ***γ***-carboxylated (active Gla) and non- ***γ***-carboxylated (inactive Gla) were produced in presence of Vitamin-K and Warfarin respectively.

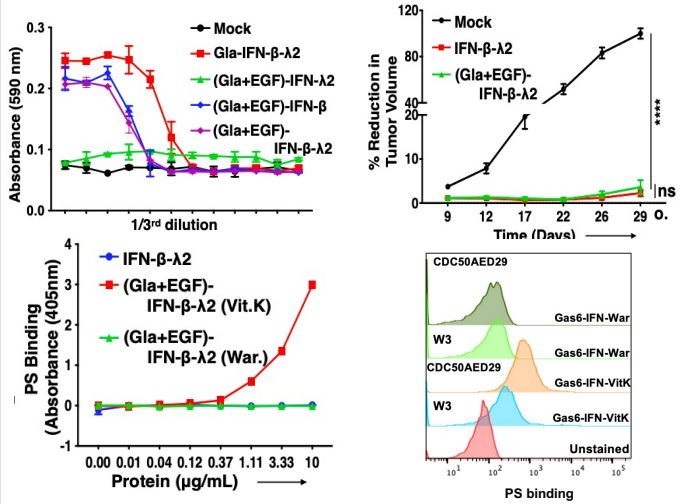


Type I and Type III IFN fusion molecules retain anti-viral activities and have enhanced anti-tumor activity



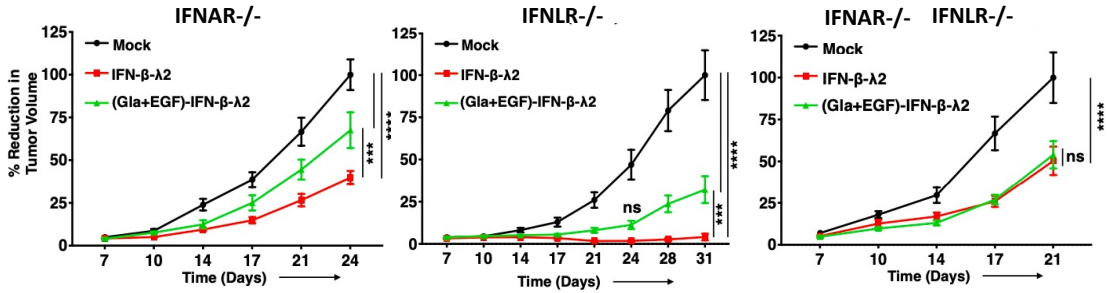
Type I and III IFN fusion proteins retain anti-viral activity to VSV in vitro assessed by crystal violet staining of protected cells (Left). EO771 cells producing fusion proteins were injected into C57BL/6 mice. Tumors producing IFN fusion proteins show enhanced anti-tumor effect (reduced tumor volume) compared to type I and III IFN alone.

Gas6-IFN-βλ-2 fusion molecules retain anti-viral activities, PS binding activities and anti-tumor activity



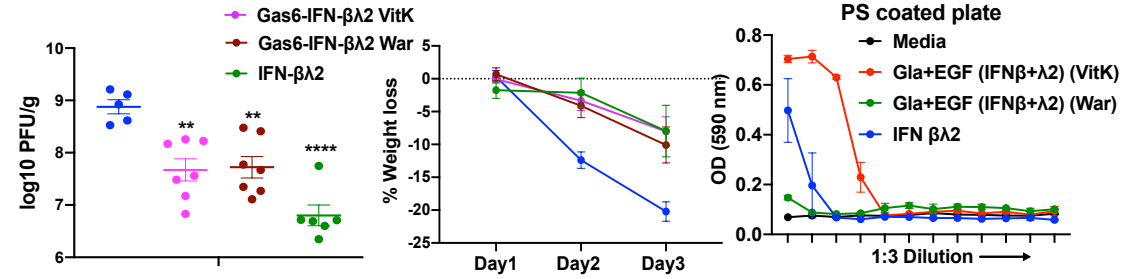
(a) Gas6-IFN-βλ-2 retains antiviral effects to VSV infection in vitro. (b) Gas6-IFN-βλ-2 has anti-tumor effect in EO771 model (c) PS ELISA shows the PS binding capability of Gas6-IFNβλ2 (Vit.K). (d) shows binding to viable, PS externalizing cells CDC50AED29.

IFN-β-λ2 and Gas6-IFN-βλ-2 have Ifnar1, Ifnlr1 independent anti-tumor effect



IFNβλ2 and Gas6-IFN-βλ-2 have anti tumor effect in tumors injected in IFNAR-/- , IFNLR-/- and IFNAR-/- IFNLR-/- mice, suggesting an IFN independent pathway.

IFN-β-λ2 and Gas6-IFN-βλ-2 have protective anti-viral effect in mouse model of Influenza infection



IFN-β-λ2 and Gas6-IFN-βλ-2 have a protective effect in Influenza infection (8h post infection) as shown by reduced viral titer (left) from lungs on day 3 after infection and protection from weight loss (center). Additionally, Gas6-IFN-βλ-2 is capable of PS targeting (right), as it shows enhanced antiviral activity on PS coated plates.

Results and conclusion

- IFN-β-λ2 and Gas6-IFN-βλ-2 are novel broad spectrum anti- tumor and anti-viral therapeutics.
- IFN-β-λ2 fusion proteins have enhanced anti-tumor and anti-viral activity than IFNβ or IFNλ2 alone.
- Gas6-IFN-βλ-2 can be used to target IFN activity to PS rich microenvironments such as tumor tissue and virus infected tissue.
- **In summary, we have engineered novel recombinant IFNs that potential as prophylactic/ therapeutic against cancer or viral infection.**

Working model