**Anti IL-17A RNA aptamer – possible therapeutic potential in some cells, more than we bargained for in others?**

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IL-17A is one of the main pathogenic factors orchestrating inflammation in diseases such as psoriasis and rheumatoid arthritis (RA) ([Martin, Towne et al. 2012](#_ENREF_5)). Although effective therapies already exist for these diseases, biologics are restricted to severely affected patients due to the considerable costs of current antibody-based therapies and potential systemic immunosuppressive and drug-induced side effects. New topical strategies are therefore required for chronic inflammation.

Aptamers are considerably cheaper to produce and furthermore are non-immunogenic. The use of stable RNA aptamers for therapy is based on their ability to fold into unique 3-dimensional structures that bind the target with high specificity and affinity. The first aptamer-based therapeutic agent, which targets vascular endothelial growth factor (VEGF) ([Huang, Moore et al. 2001](#_ENREF_3)), was FDA-approved in 2004 for treatment of macular degeneration and several other aptamers are currently in clinical trials ([Zhou, Bobbin et al. 2012](#_ENREF_10)).

Here, we have sought to show the functional efficacy of a previously described aptamer, termed Apt21-2, which blocks IL-17A binding to its receptor ([Ishiguro, Akiyama et al. 2011](#_ENREF_4)). So far, the functional properties of this aptamer have not been investigated in human pathophysiological settings. To overcome this lack of information we analysed the efficacy of Apt21-2 in a co-culture system which mimics psoriatic inflammation, using T cells isolated from healthy controls and psoriatic patients. All human samples were taken in accordance with the Declaration of Helsinki and participants gave their written informed consent (REC number: 11/YH/0368). This *in-vitro* model, using patient-derived and healthy cells, is based on our own previous work ([Muhr, Renne et al. 2010](#_ENREF_6)) with blood-derived IL-17 producers (CD4+CCR6+ T-cells) activated with TCR stimulatory antibodies (antiCD3 and antiCD28 – Biolegend, Hatfield, UK). These cells were co-cultured with healthy human primary fibroblasts. The protocols used for the culture of primary human skin cells are also based on previous work ([Wittmann, Doble et al. 2012](#_ENREF_9)). To measure the functional response a bead based ELISA assay (FlowCytomix - eBioscience, Hatfield, UK) in addition to an IL-6 and IL-8 ELISA (DuoSet human ELISA kit - RnD Systems, Abingdon, UK) was used. IL-17A (Biolegend, Hatfield, UK) mediated production of IL-6 was determined to establish the efficacy of the aptamer, as this has been employed in the previously published work ([Ishiguro, Akiyama et al. 2011](#_ENREF_4)) and we have extended this to include IL-8. It is well known that both keratinocytes and fibroblasts produce IL-6 and IL-8.

Our results confirmed that IL-6 release in recombinant IL-17A-treated fibroblasts can be significantly abrogated in a dose-dependent fashion by addition of Apt21-2 and this effect was not seen when a control aptamer (synthesised to the polymerase from foot-and-mouth disease virus, 47tr) ([Ellingham, Bunka et al. 2006](#_ENREF_2)) was used (Fig 1a). The effect was also not present if recombinant TNFα (Immunotools, Friesoythe, Germany) was used as a stimulus (Fig 1b). The same effect was confirmed for fibroblasts isolated from psoriatic patients (data not shown). Higher doses of IL-17A were used and an efficient inhibitory effect of IL-6 production could be seen in doses up to 20 ng/ml. However, the aptamer failed to consistently neutralise the effect of 40 ng/ml of rIL-17A on IL-6 production in fibroblasts. In a co-culture system of healthy fibroblasts and healthy or psoriatic CD4+CCR6+ T cells, a neutralising capacity of Apt21-2 was observed (Fig 1c). A multi-parameter experiment, using the FlowCytomix assay on the cell supernatants from the co-culture experiments was carried out. This showed that the neutralising effect of the aptamer was most prominent in reducing IL-6 levels, as shown previously ([Ishiguro, Akiyama et al. 2011](#_ENREF_4)), however a reduction was also seen in levels of IL-8 and MCP-1 (data not shown).

All experiments carried out in fibroblasts were also performed in primary human keratinocytes. However, the neutralising effect was not seen in keratinocytes and the aptamer had no effect on levels of pro-inflammatory IL-6 or IL-8 cytokine release (Fig 1d). This led us to an exploratory experiment using 3’ and 5’ labelled Apt21-2 for confocal microscopy and a 3’ labelled Apt21-2 for live cell imaging. We also included a labelled control aptamer (47tr). The results shown in Figure 2 suggest an efficient uptake of Apt21-2 and 47tr by primary human keratinocytes suggesting a non-specific uptake mechanism. The lack of availability of the aptamer in the extracellular space may well explain the absence of any counter-regulatory effect on IL-17A stimulated keratinocytes.

In summary, our experiments have confirmed the IL-17 neutralising capacity of aptamer 21-2 in fibroblast culture and also in fibroblast – T cell cultures. The best suited therapeutic approach to treat skin diseases is to give a site specific (i.e. topical) treatment reducing inflammatory mediators produced by the keratinocytes. We have shown in previous work and confirmed in this study that fibroblasts also play an active role in production of pro-inflammatory mediators. However, a topical treatment of Apt21-2 would not have neutralising effects due to aptamer uptake by upper skin layer keratinocytes. Therefore Apt21-2 would likely not be suitable for treatment of chronic inflammatory skin diseases such as psoriasis unless directly introduced into the dermis.

These results are nevertheless extremely interesting as they highlight an unrecognised function of keratinocytes which is their ability to take up RNA aptamers in a non-specific manner. Our experiments show that keratinocytes appear to take up the RNA aptamer even in the absence of its target/ligand suggesting that this process is not specific for the aptamer target molecule. It has previously been suggested that uptake of DNA by skin cells could involve macropinocytosis ([Basner-Tschakarjan, Mirmohammadsadegh et al. 2004](#_ENREF_1); [Reyes-Reyes, Teng et al. 2010](#_ENREF_8)). However, more work is needed to provide further information on the mechanisms of RNA aptamer uptake by keratinocytes and what cellular mechanisms are at play. As aptamers have the ability to specifically modulate protein-protein interactions ([Nicol, Cesur et al. 2013](#_ENREF_7)), this approach could be further utilised experimentally as well as therapeutically to target specific intracellular targets. This function of keratinocytes could be a potentially invaluable tool for targeting important pro-inflammatory signalling cascades within the epidermal cellular compartment without the need for chemical introduction of the RNA aptamer into these cells. This could be beneficial when treating inflammation involving the epidermis using a direct topical method. RNA aptamers targeting inflammatory signalling pathways could present a specific and effective topical approach which would not have the widespread side effects of systemic therapy.

Conflicts of interest

The authors state no conflict of interest.

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References

Basner-Tschakarjan, E., A. Mirmohammadsadegh, et al. (2004). "Uptake and trafficking of DNA in keratinocytes: evidence for DNA-binding proteins." Gene Ther **11**(9): 765-774.

Ellingham, M., D. H. Bunka, et al. (2006). "Selection and characterization of RNA aptamers to the RNA-dependent RNA polymerase from foot-and-mouth disease virus." RNA **12**(11): 1970-1979.

Huang, J., J. Moore, et al. (2001). "Highly specific antiangiogenic therapy is effective in suppressing growth of experimental Wilms tumors." J Pediatr Surg **36**(2): 357-361.

Ishiguro, A., T. Akiyama, et al. (2011). "Therapeutic potential of anti-interleukin-17A aptamer: suppression of interleukin-17A signaling and attenuation of autoimmunity in two mouse models." Arthritis Rheum **63**(2): 455-466.

Martin, D. A., J. E. Towne, et al. (2012). "The Emerging Role of IL-17 in the Pathogenesis of Psoriasis: Preclinical and Clinical Findings." Journal of Investigative Dermatology.

Muhr, P., J. Renne, et al. (2010). "Primary human keratinocytes efficiently induce IL-1-dependent IL-17 in CCR6+ T cells." Exp Dermatol **19**(12): 1105-1107.

Nicol, C., O. Cesur, et al. (2013). "An RNA Aptamer Provides a Novel Approach for the Induction of Apoptosis by Targeting the HPV16 E7 Oncoprotein." PLoS One **8**(5): e64781.

Reyes-Reyes, E. M., Y. Teng, et al. (2010). "A new paradigm for aptamer therapeutic AS1411 action: uptake by macropinocytosis and its stimulation by a nucleolin-dependent mechanism." Cancer Res **70**(21): 8617-8629.

Wittmann, M., R. Doble, et al. (2012). "IL-27 Regulates IL-18 binding protein in skin resident cells." PLoS One **7**(6): e38751.

Zhou, J., M. L. Bobbin, et al. (2012). "Current progress of RNA aptamer-based therapeutics." Front Genet **3**: 234.



**Figure 1** - **The neutralising effects of Apt21-2 on recombinant and T cell derived IL-17**

a: Effect of increasing concentrations of chemically-synthesised Apt21-2 (n=4) and control aptamer (n=3) on IL-6 production in IL-17A stimulated primary human dermal fibroblasts. IL-6 values for the positive control (10ng IL-17A) were set to 100 for each independent experiment to allow summary of neutralisation.

b: The ability of Apt21-2 to inhibit IL-17A versus TNFα (as a control) was tested in primary human dermal fibroblasts (n=2).

c: The neutralisation was also confirmed in the co-culture experiment (n=1).

d: Experiments as depicted in ‘a’ for human primary keratinocytes measuring IL-8 release (n=3). Mean +/- SEM bars included on a, b and d. One-way ANOVA and Bonferroni tests were performed for each group to assess statistical significance (GraphPad Prism, San Diego, CA).



**Figure 2 – Confocal microscopy to track uptake of Apt21-2 in primary human keratinocytes**

a, b: Primary human keratinocytes in the presence of Cy3 labelled Apt21-2 at 3 hours (a) and 20 hours (b), using live cell imaging.

c: Primary human keratinocytes were also treated with a Cy3 labelled control aptamer (47tr) to establish whether the uptake was aptamer specific. DAPI (top left), Cy3 (top right hand), merged (lower left).

d, e, f: Keratinocytes were also treated with a 3’Cy3 and 5’Cy5 double-labelled aptamer for 4 hours to confirm that the RNA is intact. Images were taken at increasing magnification d to f. DAPI (top left), Cy3 (top right hand), Cy5 (lower left), merged (lower right).

Cells were also treated with both 47tr and Apt21-2 in the presence of recombinant IL-17A (data not shown) and no difference in uptake was observed. Scale bars = 50 µM