Systems-biology analysis of rheumatoid arthritis fibroblast-like synoviocytes reveals cell line-specific transcription factor function

Supplementary Discussion

E2F7/E2F1 antagonistic CL2 signature

The transcriptional repressor E2F7 is the rank 3 cluster-specific TF (Fig. 1c) (q-value = 7.1 x 10^{-3}). This prediction is driven by its differential expression (3.89-fold CL2, p-value = 4.2 x 10^{-4}) (a factor in determining PPR via edge weight construction: see Methods) since the other important metric governing PPR, network connectivity, is relatively low for E2F7, as illustrated by its peripheral position and low out-degree in the CL2 TF-TF subnetwork (Fig. 3b). The transcriptional activator E2F1 (2.83-fold CL2, p-value = 5.4 x 10^{-3}), and E2F7 are mutually antagonistic and regulate proliferation, differentiation, apoptosis and responses to DNA-damage.\(^1\) However, we observe that they are both up-regulated in CL2. Paradoxically, some cancers, such as head and neck squamous cell carcinomas (HNSCCs) also exhibit the overexpression of both TFs. Mislocalization of E2F7 from the nucleus to the cytoplasm via XPO1 nuclear export is a feature in 80% of HNSCCs leading to aberrant differentiation, increased proliferation and drug resistance.\(^2\) In our data, we observe a strong correlation between E2F7 and XPO1 transcript levels (Pearson R = 0.92) indicating that, despite increased transcription of E2F7 in CL2, there is also increased XPO1 available to potentially export the E2F7 protein from the nucleus. Indeed, known transcriptional targets of E2F7 repression such as Rac GTPase activating protein 1 (RACGAP1) (2.26-fold CL2, p-value = 1.4 x 10^{-2}) and DNA-damage response genes such as RAD51\(^3\) (2.51-fold CL2, p-value = 6.8 x 10^{-3}) are significantly more highly expressed in CL2 despite the approximately 4-fold higher mRNA levels of E2F7 in CL2 compared to CL1. We regard these findings as observational and note that many other mechanisms may also “inactivate” E2F7 transcriptional effects such as post-translational modifications and mutations in the sequence of the E2F7 protein.
Supplementary References

1. Dimova, D. K. & Dyson, N. J. The E2F transcriptional network: old acquaintances with new faces. *Oncogene* **24**, 2810–2826 (2005).

2. Saenz-Ponce, N. *et al.* Targeting the XPO1-dependent nuclear export of E2F7 reverses anthracycline resistance in head and neck squamous cell carcinomas. *Sci. Transl. Med.* **10**, (2018).

3. Mitxelena, J. *et al.* An E2F7-dependent transcriptional program modulates DNA damage repair and genomic stability. *Nucleic Acids Res.* **46**, 4546–4559 (2018).
Supplementary Figures

Supplementary Figure 1

Computational and experimental pipeline overview.
**Supplementary Figure 2**

Extended representation of the Taiji Integrative Pipeline for construction of patient-specific global transcriptional gene regulation networks.
Supplementary Figure 3

(a) Hierarchical clustering of 11 OA cell lines using the top 350 TFs ranked by variance in their PPR leads to two clusters containing 7 OA patients and 4 OA patients, OA clusters 1 (OA CL1) and 2 (OA CL2) respectively, at the first split in the dendogram. 62 TFs were found to have significantly different (p-value < 0.05) PPR values from a two-tailed Students t-test between OA CL1 and OA CL2. Only 7 of which were common to the 65 differential TFs between RA CL1 and CL2 illustrating disease specificity. (b) Functional enrichment analysis of the 62 OA cluster-specific TFs results in developmental pathways including: Activation of HoX genes during differentiation (p-value = $2.47 \times 10^{-10}$ from hypergeometric test).
Supplementary Figure 4

Heatmap hierarchically clustering 245 EpiSig co-modified clusters derived from 78,598 signal-enriched 5kbp regions based on Histone ChIP-seq data for six core histone modifications.
(H3K4me1, H3K4me3, H3K9me3, H3K27ac, H3K27me3 and H3K36me3). Each column represents a single RA patient FLS cell line for a specific mark. Rows cluster into putative regulatory regions (e.g. pale green is active promoter marked by H3K4me3 and H3K27ac). Columns cluster by mark. Reading left to right, main heatmap is followed by columns: cluster id, number of 5kbp regions, distance to TSS and inter-patient variance for each mark. 10 high variance enhancer clusters marked, which map to 1,006 genes. Tables for the top significantly enriched (using Mann-Whitney test) GO Biological Process' and GO Molecular Function categories for the 1,006 genes.
a. 

b. 

cl1 tgfβ protein

c. 

cl2 tgfβ protein
Supplementary Figure 5

a. Western blot analysis of RARα knockdown efficiency in RA-FLS lines. 6 biologically independent RA-FLS cell lines were transfected with 1μg of RARα siRNA (Human RARα siRNA smartpool, Dharnacon) and control (Non-targeting Control Pool, Dharnacon) and plated for 3 days. The RARα protein expression was analyzed by Western blot, using 1:1000 dilution of RARα mouse antibody (Santa Cruz) and normalized to GAPDH (Cell Signaling). The protein level indicates 53% to 58% of RARα inhibition (p=0.004). Barplot centre line is mean and error bars +/- 0.5 s.d. Band intensities were quantified using Versadoc Quantity One 4.6.6, and the statistical significance was determined by two-tailed paired Student’s t-test.

b. TGFB protein levels by Elisa in 1%FCS/DMEM and then treated with IL-1 (2ng/ml) for CL1 and CL2 lines with (siRARA) and without (CTL) RARA knockdown. CL1 n=4 and CL2 n=4
biologically independent cell lines. Red centre line for median, whiskers represent maximum and minimum values, box width from quartile 1 to quartile 3.

c. CDKN2B western blot performed once for CL1 and CL2 (CL1 n=3 and CL2 n=3 biologically independent cell lines) treated with IL1 IL-1 (2ng/ml) with (siRARA) and without (CTL) RARA knockdown.

d. Phospho SMAD2/3 by Elisa with and without TGFB treatment for CL1 and CL2 lines. CL1 n=4 and CL2 n=4 biologically independent cell lines. Red centre line for median, whiskers represent maximum and minimum values, box width from quartile 1 to quartile 3.

Source data are provided as a Source Data file.
Supplementary Figure 6

The RARA ligand ATRA has differential effects on CL1 and CL2 FLS growth. CL1 at Day 7 exhibited increase in proliferation under ATRA (p=0.0315 by two-tailed paired Student’s t-test). FLS were cultured with 1 uM of ATRA in either medium or 10 ng/ml of PDGF. Cell growth was quantified using an MTT assay. *: p<0.05
Supplementary Figure 7

Heatmap as per Figure S4 legend. 2 enhancer clusters with significant CL1 vs CL2 difference in EpiSig signal marked, which map to 242 genes. Functional enrichment analysis of the 242 genes.
## Supplementary Tables

| Taiji prediction                                                                 | Experimental validation                                                                 |
|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| **Molecular events**                                                             |                                                                                        |
| RARα binding CL1 > CL2 at RARα binding motif                                      | Confirmed with ChIP PCR at TCIRG1 promoter                                              |
| RARα deficiency: differential effect on CL1 and CL2 TGFβ mRNA levels             | Confirmed for TGFβ by RT-qPCR                                                           |
| RARα deficiency: differential effect on CL1 and CL2 TGFβ regulates               | Confirmed for CDK2NB, ROCK1, CCND1 expression by RT-qPCR.                                |
| RARα deficiency: differential effect on CL1 and CL2 TGFβ regulates               | Confirmed for CDK2NB protein by Western Blot                                            |
| Normal TGFβ signaling in CL1 and CL2                                             | Confirmed using P-SMAD assay after stimulating cells with TGFβ. Showed that explanation for decreased TGFβ regulates was not due to deficient signaling |
| RARα deficiency: differential effects on EMT markers in CL1 and CL2              | Confirmed based on FN1 and VIM expression by RT-qPCR                                    |
| **FLS functions**                                                                |                                                                                        |
| RARα deficiency: differential effect on cell invasion in CL1 and CL2             | Confirmed in Matrigel assay                                                           |
| RARα deficiency: differential effect on cell proliferation in CL1 and CL2         | Confirmed by MTT assay                                                                 |
| CL1 and CL2 cells phenotypically different.                                      | Confirmed by cell size analysis using flow cytometry and image analysis (data not shown) |
| Proliferation of CL1< CL2                                                        | Confirmed by cell counting in culture                                                   |
| RARα agonist (ATRA): differential effects on CL1 and CL2 proliferation           | Confirmed by MTT assay                                                                 |

## Supplementary Table 1

Summary of computational predictions and experimental validation.
| Marker                          | %  | stddev |
|--------------------------------|----|--------|
| CD34+                          | 2.0| 0.8    |
| CD90+                          | 95.9| 2.4   |
| FAPα+                          | 90.5| 4.9    |
| CD14+                          | 2.0| 1.1    |
| PDPN+                          | 92.0| 2.3    |
| CD90+, FAPα+, PDPN+            | 82.5| 6.7    |
| CD90+, CD14+, PDPN-            | 1.8| 1.0    |

**Supplementary Table 2**

Flow data on FLS phenotype.