Increased expression of Ectodysplasin A2 Receptor EDA2R is the most remarkable and ubiquitous aging-related transcriptional hallmark

Maria Chiara Barbera

Mario Negri Institute for Pharmacological Research

Laura Di Rito

Mario Negri Institute for Pharmacological Research

Luca Guarrera

Mario Negri Institute for Pharmacological Research <https://orcid.org/0000-0001-7475-3345>

Ilaria Craparotta

Mario Negri Institute for Pharmacological Research

Arianna Vallerga

INSTITUTE OF ONCOLOGY RESEARCH (IOR)

Margherita Romeo

Institute of Clinical Chemistry and Laboratory Diagnostic Medical Faculty, Heinrich Heine University and the IUF-Leibniz Research Institute for Environmental Medicine <https://orcid.org/0000-0002-3020-8545>

Sarah Mapelli

Humanitas Clinical and Research Center-IRCCS <https://orcid.org/0000-0002-0129-2055>

Marco Bolis (✉ marco.bolis@ior.usi.ch)

INSTITUTE OF ONCOLOGY RESEARCH (IOR) <https://orcid.org/0000-0003-4377-5079>

Brief Communication

Keywords:

Posted Date: August 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-774469/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

ARTICLE TYPE: **Brief Communication**

TITLE: Increased expression of Ectodysplasin A2 Receptor EDA2R is the most remarkable and ubiquitous aging-related transcriptional hallmark

TOTAL WORD COUNT: 1550

ABSTRACT: 70

MAIN TEXT: 549

FIGURE LEGENDS: 538

REFERENCES: 393

Graphical Abstract

TITLE: Increased expression of Ectodysplasin A2 Receptor EDA2R is the most remarkable and ubiquitous aging-related transcriptional hallmark

M. Chiara Barbera^{1*}, Laura Di Rito^{1*}, Luca Guarrera^{1*}, Ilaria Craparotta¹, Arianna Vallergera^{2,3,4}, Romeo M.⁶, Sarah N. Mapelli⁵, Marco Bolis^{1,2,3,4,@}

* These authors contributed equally to this work

@ Correspondence to: marco.bolis@ior.usi.ch, marco.bolis@marionegri.it

¹Computational Oncology Unit, Department of Oncology, 'Mario Negri' Institute for Pharmacological Research IRCCS, Via Mario Negri 2, 20156 Milano, Italy

²Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Bellinzona, TI 6500, Switzerland

³Università Della Svizzera Italiana (USI), Faculty of Biomedical Sciences, Lugano CH-1011, Switzerland.

⁴Swiss Institute of Bioinformatics, Bioinformatics Core Unit, Bellinzona, TI 6500, Switzerland

⁵Humanitas Clinical and Research Center-IRCCS, Milan, Italy.

⁶Institute of Clinical Chemistry and Laboratory Diagnostic Medical Faculty, Heinrich Heine University and the IUF-Leibniz Research Institute for Environmental Medicine, Düsseldorf, 40225 Germany

ABSTRACT

We report that increased mRNA-expression of Ectodysplasin-A2-Receptor (EDA2R) is the most remarkable aging-associated transcriptional perturbation in humans, and that its induction is confirmed in murine-models of Hutchinson-Gilford progeria syndrome and in other species. Up-regulation of EDA2R occurs in all solid-tissues and is complemented by the progressive transcriptional increase of its ligand in bloodstream. Strengthening of EDA2R/EDA-A2 signaling may exacerbate inflammation in ageing individuals which might be prevented by EDA2R/EDA-A2 blockade.

MAIN TEXT

Ectodysplasin A2 Receptor is part of the tumor necrosis factor receptor (TNFR) superfamily which selectively binds to *EDA-A2*, a protein encoded by an alternative splicing isoform of *EDA* (Ectodysplasin A) gene. *EDA2R* receptor has been cataloged as a target of *TP53*^{1,2} and EDA2R/EDA-A2 signaling has been observed to mediate activation of JNK, NF- κ B pathways^{3,4} and to promote inflammation, apoptosis and cell death^{2,5-9}. Indeed, in diabetic kidneys, *EDA2R* has been shown to mediate podocyte injury through ROS generation¹⁰. Additionally, several studies indicated that the gene locus of *EDA2R* is associated with age-associated androgenetic alopecia (AGA), which is a known common heritable polygenic disorder^{11,12}. Moreover, *EDA2R* mRNA expression was reported to be elevated in the aging lungs¹³.

To identify genes whose expression could be positively correlated with the increasing age of individuals, our group selectively screened the Genotype-Tissue Expression database (GTEx)¹⁴ looking for transcriptional changes occurring in a tissue-independent fashion. To this purpose, we went through a rigorous multi-step bioinformatics analysis procedure which aimed at minimizing possible confounding factors such as gender

differences or the precise anatomical sub-localization of the sample of origin (See Methods section). *EDA2R* emerged as the most noticeable outlier of the analysis, as its expression resulted to be strikingly well correlated to the increasing age of donors in a tissue-type independent manner. Indeed, *EDA2R* appeared among the very top correlated genes in all solid tissues evaluated (**Fig1A, Supp.Fig1A, Supp.Table1**) and its median correlation coefficient ranked first in the integrated pan-tissue analysis (**Fig1B**). Furthermore, strong induction of *Eda2r* also emerged in aging tissues of rats and mice, suggesting a non-species-specific behavior (**Fig1A-B, Supp.Fig1B, Supp.Table2**). Next, to further strengthen our findings, we investigated transcriptional changes occurring in murine models of Hutchinson-Gilford progeria syndrome (HGPS), a recognized proxy of accelerated aging in humans, to determine if *Eda2r* induction could be peculiar to the disease. Remarkably, the analysis revealed *Eda2r* itself as one of the very top genes being most significantly up-regulated in the aorta of HGPS mice compared to the age matched wild-type counterpart (2 months old), confirming thereby a deep connection to the aging processes (**Fig2A-B, Supp.Table3**). Altogether, our findings strongly support a unique tissue-independent association between aging and *EDA2R* mRNA levels.

Conversely, transcription from the *EDA* locus, which codes for the *EDA2R* ligand, did not correlate substantially with age in most solid tissues, however, age-mediated induction of *EDA* turned evident in blood-derived samples (**Fig3A**), showing a different behavior compared to *EDA2R* receptor. In particular, the *EDA-A2* isoform resulted to be more expressed in venous blood compared to the *EDA-A1* counterpart (**Supp.Fig1C, Supp.Table1**), being the former the selective ligand of *EDA2R*. As increased *EDA-A2/EDA2R* mediated signaling could trigger inflammatory responses, we questioned whether baseline activity of inflammatory pathways would also correlate to age in a tissue-independent manner. Our observations confirmed this last hypothesis in most tissues (**Fig3B**), indicating inflammation as one of the top-ranking biological process associated

with aging, suggesting thereby a possible link with EDA2R activity.

Taken together, our results disclose a single-gene biomarker, impressively associated to increasing age in every individual tissue, solving a scientific inquiry which has been previously answered exclusively with DNA methylation models^{15,16}. More importantly, the EDA2R/EDA-A2 axis, with its ligand-receptor activity and downstream signaling pathways, suggest novel targets for the development of compounds aimed at slowing down chronic inflammation and consequent age-associated decline.

Author Contributions

M.B., C.B., and S.M. originally developed the concept and further elaborated on it. M.B., L.D.R., and L.G. carried out all bioinformatic analyses. M.B., S.M., I.C., M.R., wrote the manuscript and designed all figures.

Declaration of Interests

The authors declare no conflicts of interest.

Correspondence

Correspondence and requests should be addressed to M.B., (marco.bolis@ior.usi.ch ; marco.bolis@marionegri.it)

122 **Figure 1**

Figure 1

***EDA2R* remarkably associates with age and is maintained across species.**

A) Boxplots representing Pearson's correlation coefficients between mRNA expression of all quantified human genes with sample age, as determined from the GTEx collection of human tissue samples (RNA-Seq). Tissues were evaluated separately as indicated. The yellow dot indicates the relative positioning of *EDA2R* as obtained by ranking genes by their correlation coefficient with donors' age. In several tissues, *EDA2R* scores first being the most correlated gene, and in most scenarios, its ranking is among the top 10 genes. For ranking positions exceeding the 50th, the reference quartile is reported, with Q1 indicating the highest quartile

and Q4 the lowest referred to each tissue. **B)** Representation of the median Pearson's correlation coefficient for each gene (dots) in all solid tissues. Red dots represent genes increasing with age (positive median correlations), blue represent genes decreasing with age (negative median correlations). Yellow dot indicates *EDA2R*, which scores as the top ranking gene having high correlation coefficients in all tissues considered. **C) Left**, RMA normalized expression of *Eda2r* measured by microarrays (intensities). Boxplots compare murine gastrocnemius samples derived from young and old mice (respectively 10 weeks and 24 months; 3 replicates in each group). Adjusted p-value (q-value) is indicated and conventionally represented with ** if minor than 0.01. **Right**, The panel shows significance levels ($-10 \times \log_{10} \text{FDR}$) for all genes. *Eda2r* relative positioning is indicated in yellow. **D)** Changes in *Eda2r* expression ($\log_2$ fold-change) in rat gastrocnemius comparing older individuals versus 6 months old rats (ages are reported below each bar). Significance is indicated with FDR adjusted p-value (q-value) and represented with *** if minor than 0.001, not significant (ns) if major than 0.05.

147 **Figure 2**
148

Figure 2

150 ***Eda2r* is among the most upregulated genes in murine models of Hutchinson-**
151 ***Gilford progeria syndrome (HGPS).*** **A)** Volcano plot representing changes in the gene
152 **expression of murine aortic artery comparing HGPS, 2 months old mice (LMNA-G609G**
153 **mutant) against wild type, 2 months old age-matched mice. Log₂ fold-change and**
154 **significance (-10xlog₁₀FDR) are represented. In yellow, as the third most significant**
155 **differentially expressed gene, *Eda2r*, which is strongly upregulated in the HGPS group. **B)****
156 **Boxplots representing *Eda2r* expression in wild type and HGPR mice (LMNA-G609G) (6**
157 **replicates in each group). Significance is reported with FDR-adjusted p-value (q-value)**
158 **and conventionally represented with *** if minor than 0.001.**
159

160

Figure 3**a** **b** **Figure 3**

162

163

164

Age positively correlates with *EDA* (*EDA2R* ligand) in human blood and ageing associates with up-regulation of inflammation-related gene sets in human tissues.

165

A) Violin plot representing Pearson's correlations between expression and age for all human genes in blood (GTEx dataset). Yellow dot represents *EDA2R* (ranking within the first quartile), magenta dot indicates the *EDA* associated correlation coefficient. Coefficients (r) and adjusted p-values (q -values) are reported for both genes. While *EDA2R* shows less correlation compared with its behavior in solid tissues, *EDA* is among the most positively correlated genes.

166

167

168

169

170

171

172

173

174

175

B) Aging-dependent perturbations of Hallmark and Reactome inflammation-related gene sets in all human tissues. Circle dimensions are proportional to statistical significance expressed as $-10 \times \log_{10}(\text{FDR})$, colors represent positive (red) and negative (blue) correlations. Gene-sets reaching showing $\text{FDR} < 0.05$ are depicted with high saturation, not significant genes with low saturation. While inflammatory pathways increase with age in most tissues, a decrease is observed in selective tissues like liver and pancreas.

References

- 176 1 Tanikawa, C., Ri, C., Kumar, V., Nakamura, Y. & Matsuda, K. Crosstalk of EDA-
177 A2/XEDAR in the p53 signaling pathway. *Mol Cancer Res* **8**, 855-863,
178 doi:10.1158/1541-7786.MCR-09-0484 (2010).
- 180 2 Brosh, R. *et al.* p53-dependent transcriptional regulation of EDA2R and its
181 involvement in chemotherapy-induced hair loss. *FEBS Lett* **584**, 2473-2477,
182 doi:10.1016/j.febslet.2010.04.058 (2010).
- 183 3 Sinha, S. K., Zachariah, S., Quinones, H. I., Shindo, M. & Chaudhary, P. M. Role of
184 TRAF3 and -6 in the activation of the NF-kappa B and JNK pathways by X-
185 linked ectodermal dysplasia receptor. *J Biol Chem* **277**, 44953-44961,
186 doi:10.1074/jbc.M207923200 (2002).
- 187 4 Hayden, M. S. & Ghosh, S. Regulation of NF-kappaB by TNF family cytokines.
188 *Semin Immunol* **26**, 253-266, doi:10.1016/j.smim.2014.05.004 (2014).
- 189 5 Song, L. *et al.* Macrophage-derived EDA-A2 inhibits intestinal stem cells by
190 targeting miR-494/EDA2R/beta-catenin signaling in mice. *Commun Biol* **4**,
191 213, doi:10.1038/s42003-021-01730-0 (2021).
- 192 6 Verhelst, K. *et al.* XEDAR activates the non-canonical NF-kappaB pathway.
193 *Biochem Biophys Res Commun* **465**, 275-280, doi:10.1016/j.bbrc.2015.08.019
194 (2015).
- 195 7 Kwack, M. H., Kim, J. C. & Kim, M. K. Ectodysplasin-A2 induces apoptosis in
196 cultured human hair follicle cells and promotes regression of hair follicles in
197 mice. *Biochem Biophys Res Commun* **520**, 428-433,
198 doi:10.1016/j.bbrc.2019.10.031 (2019).
- 199 8 Chang, B., Punj, V., Shindo, M. & Chaudhary, P. M. Adenoviral-mediated gene
200 transfer of ectodysplasin-A2 results in induction of apoptosis and cell-cycle
201 arrest in osteosarcoma cell lines. *Cancer Gene Ther* **14**, 927-933,
202 doi:10.1038/sj.cgt.7701078 (2007).
- 203 9 Yang, L., Huang, X., Wang, W., Jiang, T. & Ding, F. XEDAR inhibits the
204 proliferation and induces apoptosis of gastric cancer cells by regulating JNK
205 signaling pathway. *Biosci Rep* **39**, doi:10.1042/BSR20192726 (2019).
- 206 10 Lan, X. *et al.* EDA2R mediates podocyte injury in high glucose milieu. *Biochimie*
207 **174**, 74-83, doi:10.1016/j.biochi.2020.04.003 (2020).
- 208 11 Prodi, D. A. *et al.* EDA2R is associated with androgenetic alopecia. *J Invest*
209 *Dermatol* **128**, 2268-2270, doi:10.1038/jid.2008.60 (2008).
- 210 12 Richards, J. B. *et al.* Male-pattern baldness susceptibility locus at 20p11. *Nat*
211 *Genet* **40**, 1282-1284, doi:10.1038/ng.255 (2008).
- 212 13 de Vries, M. *et al.* Lung tissue gene-expression signature for the ageing lung in
213 COPD. *Thorax*, doi:10.1136/thoraxjnl-2017-210074 (2017).
- 214 14 Carithers, L. J. *et al.* A Novel Approach to High-Quality Postmortem Tissue
215 Procurement: The GTEx Project. *Biopreserv Biobank* **13**, 311-319,
216 doi:10.1089/bio.2015.0032 (2015).
- 217 15 Horvath, S. DNA methylation age of human tissues and cell types. *Genome Biol*
218 **14**, R115, doi:10.1186/gb-2013-14-10-r115 (2013).
- 219 16 Hannum, G. *et al.* Genome-wide methylation profiles reveal quantitative views
220 of human aging rates. *Mol Cell* **49**, 359-367, doi:10.1016/j.molcel.2012.10.016
221 (2013).

Methods

Data analysis workflow: RNA-Seq raw counts data were retrieved from GTEx v8¹. 17382 samples from 980 healthy individuals, derived from the whole variety of tissues included within this transcriptional collection of samples were parsed. Analyses were performed in R statistical environment and sequencing counts were normalized and further processed using the variable stabilizing transformation (VST) algorithm embedded within the *DESeq2* package². We focused at removing batch effects prior to performing tissue-specific correlation to age.

Batch effects corrections and transcriptional correlations to age: Batch effects were explored based on Principal Component analyses (PCA), which were performed separately for every tissue (the top 1000 most variable genes were used for the dimensionality reduction). Being gender and the anatomical sub-localization within the same tissue-type (i.e., Brain region) the most prominent sources of variability, data were corrected for these categorical variables using the *removeBatchEffect* algorithm, included in the R package *limma*³. Importantly, when adjusting for these features, we defined the biological age of the donor as the batch covariate in the design matrix, making thereby sure that age-related differences would not be biased by batch corrections.

Subsequently, for each tissue individually, we computed Pearson's correlations between gene expression and age (represented in years) for all available samples. We derived p-values associated to correlation coefficients and for each tissue considered, we adjusted p-values for multiple testing using the false discovery rate (FDR) method.

Gene-set enrichment analysis

Briefly, for each tissue, we ranked genes based on the adjusted p-value resulting from their correlation with the age of individuals. Gene-set enrichment analysis was performed

using *Camera*⁴ on Hallmarks (MSigDB) and Reactome gene-sets.

Data sources and reprocessing

RNA-Seq data for human samples were retrieved from GTEx database and processed as described in the Data analysis workflow section. Gene expression data (Affymetrix Mouse Genome 430 2.0 Array) for the gastrocnemius muscle of young (10 weeks) and old (24 months) mice were obtained from GSE52550. Data were reprocessed from raw files using the *affy* package⁵ and normalized using the Robust Multi-Array expression measure using sequence information provided by the *gcrma* package⁶. Downstream differential expression was performed using *limma*. RNA-Seq data for aging rat tissues were retrieved from SRA project PRJNA516151. Differential expression results of all genes are provided by the authors and are accessible in form of supplementary files from the respective publication⁷. RNA-Seq data for LMNA-G609G murine models of Hutchinson-Gilford Progeria Syndrome (HGPS) were retrieved from GSE165409. Sequencing reads from raw *fastq* files were aligned to the GRCm39 release of the reference mouse genome (primary assembly) and annotated using the latest version Gencode GTF file (M27). Reads counts were then processed in R Statistical environment and downstream analysis was performed through the DESeq2 pipeline.

References (METHODS)

- 1 Carithers, L. J. *et al.* A Novel Approach to High-Quality Postmortem Tissue Procurement: The GTEx Project. *Biopreserv Biobank* **13**, 311-319, doi:10.1089/bio.2015.0032 (2015).
- 2 Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**, 550, doi:10.1186/s13059-014-0550-8 (2014).
- 3 Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* **43**, e47, doi:10.1093/nar/gkv007 (2015).
- 4 Wu, D. & Smyth, G. K. Camera: a competitive gene set test accounting for inter-gene correlation. *Nucleic Acids Res* **40**, e133, doi:10.1093/nar/gks461 (2012).
- 5 Gautier, L., Cope, L., Bolstad, B. M. & Irizarry, R. A. affy--analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* **20**, 307-315, doi:10.1093/bioinformatics/btg405 (2004).
- 6 Gharaibeh, R. Z., Fodor, A. A. & Gibas, C. J. Background correction using dinucleotide affinities improves the performance of GCRMA. *BMC Bioinformatics* **9**, 452, doi:10.1186/1471-2105-9-452 (2008).
- 7 von Kleeck, R. *et al.* Arterial stiffness and cardiac dysfunction in Hutchinson-Gilford Progeria Syndrome corrected by inhibition of lysyl oxidase. *Life Sci Alliance* **4**, doi:10.26508/lisa.202000997 (2021).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigures.pdf](#)
- [SuppTable1.xlsx](#)
- [SuppTable2.xlsx](#)
- [SuppTable3.xlsx](#)