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RESEARCH ARTICLE

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The human-specific noncoding RNA *RP11-424G14.1* functions at the intersection of sexually dimorphic pathways in inflammation, senescence, and metabolism

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Abstract

Sexual dimorphism is a fundamental characteristic of various physiological and pathological processes in humans, including immune responses, senescence, and metabolism. Most studies on the sex bias have focused on sex hormones or female-biased genes, whereas male-biased genetic factors remain understudied. Here, we show that the Y-linked noncoding RNA, *RP11-424G14.1*, is expressed in human male keratinocytes. Microarray study suggests the NF-κB pathway as the top biological pathway affected by *RP11-424G14.1* knockdown, consistent with known sex differences in inflammation. Additionally, *IGFBP3* is identified as the top gene supported by *RP11-424G14.1* in male keratinocytes. Conversely, in female keratinocytes, *IGFBP3* is the top gene repressed by the X-linked long noncoding RNA *XIST*, suggesting a central role of *IGFBP3* in mediating sexual dimorphism. Knockdown of *RP11-424G14.1* or IGFBP3 in male keratinocytes inhibits cellular senescence, consistent with increased longevity in females. IGFBP3 expression is dependent on insulin, and metabolomics analysis suggests that *RP11-424G14.1* and IGFBP3 regulate acrylcarnitine metabolism. Our study identifies the role of the *RP11-424G14.1*-IGFBP3 pathway in coordinating sex differences in immunity, senescence, and metabolism. With *RP11-424G14.1* being a human-specific genetic element, our study suggests the evolving feature of sexual dimorphisms in biological processes.

KEYWORDS

inflammation, metabolism, noncoding RNA, senescence, sexual dimorphism

1 | INTRODUCTION

Sexual dimorphism is a fundamental characteristic of various physiological and pathological processes in humans. Generally, it is accepted that females mount stronger innate and adaptive immune responses than males, which is supported by the clinical observation of strikingly increased prevalence of autoimmune diseases in females.[1–3] In human populations, women are known to live longer than men, consistent with lower biological ages as assessed by molecular markers.[4,5] Furthermore, sex differences have been identified in many aspects of energy

balance regulation including glucose metabolism and insulin sensitivity as well as lipid storage and utilization patterns, which may influence the pathogenesis of diseases including obesity and diabetes.[6,7]

Studies on the molecular mechanisms underlying sex differences in human physiology have been focused on sex hormones.[8–10] Additionally, gene-dosage effects of the X- and Y-chromosomes have been described and on the single-gene level, sex-biased, protein-coding genes have been identified with roles in regulating sexually dimorphic processes.[11–14] The map of sexually dimorphic genetic elements is just starting to unfold, as protein-coding regions only make up approximately 1 percent of the human genome and the rest 99 percent is non-coding.[15] The active transcription of non-protein coding genes gives rise mainly to long non-coding RNA (lncRNAs, 80%–90%).[16]

Long non-coding RNA (lncRNA) are typically transcripts longer than 200 nt that do not code for proteins but may still serve a diverse array of functions.[17] They may regulate expression epigenetically by coordinating with DNA or histone-modifying enzymes or their products and can also directly affect transcription by interacting with or acting as transcription factors. Post-transcriptionally, lncRNA can inhibit or coordinate with small interfering (siRNA) or microRNA (miRNA).[18] Though not as common, they are also capable of post-translational modification.[19]

The importance of lncRNA in sex-biased expression has only started to gain traction in the last decade. One of the most studied in this regard is X-inactive specific transcript, *XIST*, known for its role in female development through X-chromosome inactivation (XCI).[20] During XCI, *XIST* RNA triggers gene silencing and drives a major structural reorganization to shut down one copy of the X-chromosome, which encodes for various immune-associated genes, in females. *XIST* overexpression has been associated with systemic autoimmune disease including systemic lupus erythematosus (SLE) and rheumatoid arthritis, both of which are female-biased.[21] Beyond immune regulation, lncRNA is implicated in both glucose and lipid metabolism, with the focus being *XIST* as well.[22] To the best of our knowledge, little is known about the function of other lncRNAs in mediating sexual dimorphism.

To this end, in this study we have address the expression and function of *RP11-424G14.1*, a lncRNA of 2666 bp length encoded by the Y chromosome. Intriguingly, it is also a human-specific genetic elements and does not show homology in alignment with the rhesus monkey, mouse, or other sequenced vertebrates. We confirm the male-specific expression of *RP11-424G14.1* in human keratinocytes and identify its multifaceted function in mediating sex differences in inflammation, senescence, and metabolism.

2 | MATERIALS AND METHODS

2.1 | Keratinocyte culture and transfection

ATCC primary epidermal keratinocyte cultures were grown in Keratinocyte Growth Medium, or KGM, (Lifeline Cell Technology) on labeled 10 cm cell culture-treated dishes. KGM was refreshed after seeding and every other day after during culture. Upon sufficient cell growth and 70%–90% cell confluency, keratinocytes were collected via trypsinization, counted by hemocytometer, and transfected on the Lonza 4-D X unit nucleofector according to P3 Lonza keratinocyte nucleofection protocols (V4XP-3024). If the cells were treated with immunostimulants, they (and their controls) were given 24 h post-nucleofection to recover. 24 h post-treatment, cells were either harvested for RNA or protein, or were collected and used for experiments. siRNA used was purchased through Thermofisher: *Silencer* Select negative control siRNA (referred to as scrambled siRNA), pre-designed siRNAs for *XIST* (assay ID: n272634) or IGFBP3 (assay ID: s7228), or custom designed for RP11-424G14.1 (GGGAAGUGUUUGUACAGUATT and UACUGUACAAACACUUCCCGA).

2.2 | qPCR

RNA isolated from keratinocyte culture either by Trizol extraction or Miniprep was quantified and used to make cDNA with reverse transcriptase. Standardized amounts of cDNA template were pipetted into 96-well plates with respective primers, Power SYBR Green master mix, and nuclease-free water. Optical adhesive film was used to cover the plates, then centrifuged and run on a QuantStudio 6 at standard speed for 40 cycles. Differential gene expression was calculated using the ΔΔct method.

2.3 | Microarray

mRNA was isolated from cultured male and female keratinocytes using Trizol following manufacturer instructions. mRNA was hybridized to the Thermofisher human Clariom S array. Raw signals were obtained by the GeneChip Scanner and analyzed by the Transcriptome Analysis Console Software according to standard

procedures. Differentially expressed genes were identified using a false discovery rate of 0.05 and a fold change of 2.

2.4 | Metabolomics

Keratinocyte lysates were profiled using Biocrates MxP Quant500 plates with LC- and FIA-MS following manufacturer instructures. Metabolites were identified, quantified, and normalized by the MetIDQ workflow following standard analysis workflows. Differentially expressed metabolites were identified using an adjusted *p*-value of 0.05.

2.5 | Immunofluorescence staining and imaging

Keratinocytes were transferred to 8-well chamber slides after transfection at a seeding density of 70,000–100,000 per well. After control or target gene knockdown such as XIST, RP11-424G14.1, or IGFBP3 knockdown (oligo info specified in the 'keratinocyte culture and transfection' section), cells were washed with PBS, fixed with 4% paraformaldehyde (PFA) for 15 min, and permeabilized with 0.1% Triton X-100 for 5 min. Cells were then washed in wash buffer (PBS with 0.1% Triton X-100) for 5 times and stained with the respective primary antibodies for 1 h at room temperature at manufacturer-recommended dilutions (IGFBP3, Thermofisher, 10,189-2-AP; NFkB, Cell Signaling Technologies, 3033; pIkBa, Cell Signaling Technologies, 2859; p21, Cell Signaling Technologies, 2947; THBS2, Thermofisher, MA5-46953; TSPAN1, Thermofisher, PA5-119458; WNK1, Abcam, ab174854), followed by 5 washes. Cells were subsequently stained the respective secondary antibodies at manufacturer-recommended dilutions (goat anti-rabbit IgG (H+L) Alexa Fluor 488, Thermofisher, A11008; donkey anti-mouse IgG (H+L) Alexa Fluor 488, Thermofisher, A21202) for 1 h. Cells were then washed with milliQ water, stained with 1 μg/mL DAPI for 5 min, and washed again. Upon completion of staining, the cells were then covered with Vectashield antifade mounting medium and imaged on a Nikon TI-2 E inverted fluorescent microscope. The same acquisition parameters, including laser intensity and exposure settings, were used for all conditions in comparison.

2.6 | Western blot

Keratinocytes were transferred to 6-well tissue culture-treated plates after transfection. After treatments, cells were washed with ice-cold PBS and placed on ice. Cells were then treated with RIPA buffer and scraped to isolate protein. Cell-lysate protein concentration was measured for each sample using a bicinchoninic acid assay with bovine serum albumin for a standard on an Agilent BioTek Epoch plate reader and Take3 plate. For each sample, 35 μL were loaded into wells on a precast mini-PROTEAN gel, containing 20 μg protein, 8.75 μL 4× Laemmli buffer, and milliQ water. 4 μL of BioRad Protein Precision Plus standard ladder were loaded into surrounding wells. Gels were run in a BioRad mini-PROTEAN tetra cell with Tris-glycine-SDS running buffer at 100 V for 2 h. Upon separation, gels were then equilibrated and transferred to an activated PVDF membrane for blotting, according to the BioRad transfer manual. Blots were then cut based on ladder-indicated size ranges to capture protein targets for efficient antibody probing. Blots were rinsed in Tris buffered saline with 0.1% Tween-20 (TBS-T) and then blocked in 5% non-fat dry milk in TBS-T for 1 hour at RT. Primary and HRP-linked secondary antibodies were diluted to indicated concentrations. Blots were incubated with indicated primary antibodies for 1 h at RT and then washed 3x in TBS-T. Blots were then stained with respective secondary antibodies for 1 h at RT and then washed 5x in TBS-T and 1x in TBS. Stained blots were marked with WesternSure pen to identify marker lanes upon imaging. Stained blots were incubated with LI-COR WesternSure chemiluminescent substrate for 5 min and placed in a laminated pouch, aspirating any excess solution. Blots were imaged on a C-Digit, after 12-min exposure.

3 | RESULTS

3.1 | Sex-biased expression of lncRNAs in human keratinocytes

To understand the potential roles of lncRNAs in regulating sexual dimorphism in human male keratinocytes, we used qPCR to examine the expression levels of top male-biased lncRNAs suggested by previous RNA-sequencing studies using whole skin biopsies.[14] Of the three candidates tested, including *RP11-372M18.2*, *RP11-424G14.1*, and *RP11-331F4.1*, we were able to detect male-specific expression of *RP11-424G14.1* in keratinocytes (Figure 1A). It is possible that the other two candidates, *RP11-372M18.2* and *RP11-331F4.1*, are expressed in other types of skin cells such as fibroblasts instead. Consistent with its male-specific expression, *RP11-424G14.1* maps to chromosome Y: 21,853,827–21, 856, 492 with no overlap to known protein-coding genes. The expression level of *RP11-424G14.1*, but not the control gene *GAPDH*, was significantly reduced by knockdown using its sequence-specific siRNA, suggesting specificity of expression detection (Figure 1B, Figure S1). We did not detect a significant association between *RP11-424G14.1* and the age of donors (Figure 1A).

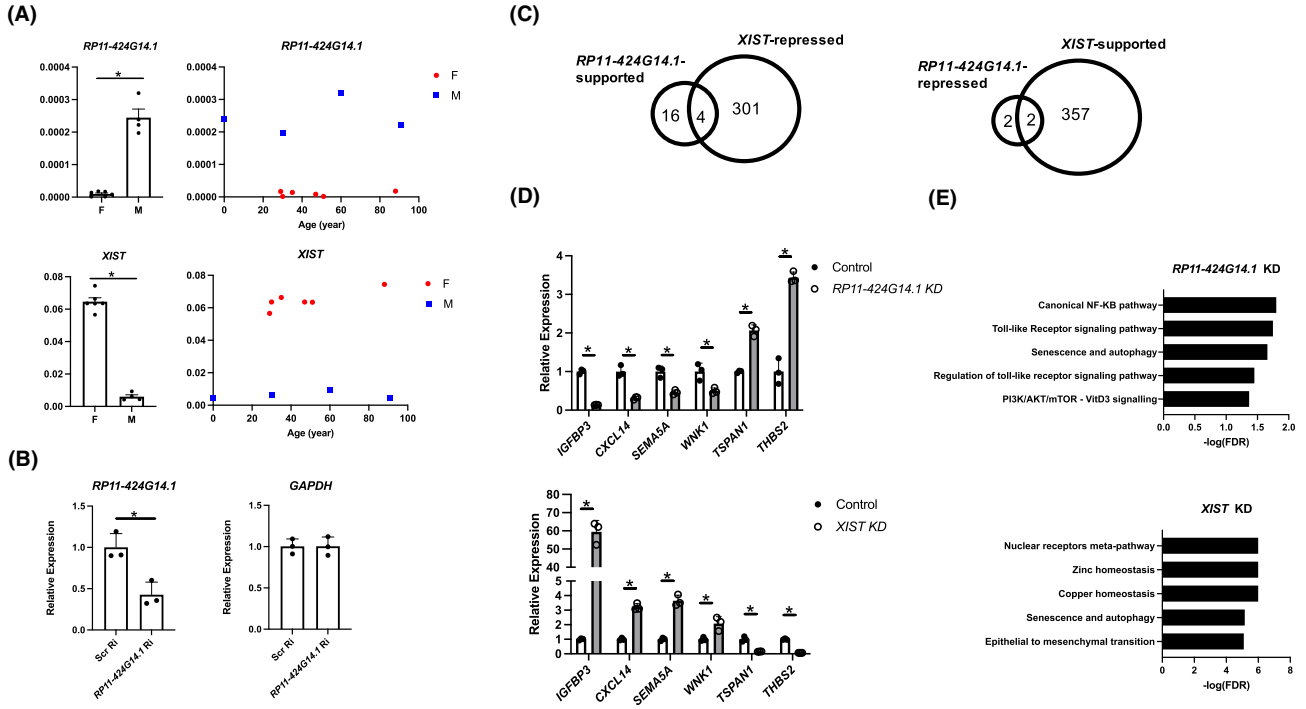

FIGURE 1 Sex-biased expression of *RP11-424G14.1* and *XIST* as well as their downstream targets. (A) Expression levels *RP11-424G14.1* and *XIST* in female (F) and male (M) keratinocytes (left) by qPCR, showing lack of association with age (right). (B) Specificity of *RP11-424G14.1* detection and knockdown, shown by expression levels of *RP11-424G14.1* and GAPDH upon scrambled RNAi (Scr Ri, as control) or RP11-424G14.1 sequence-specific RNAi by qPCR. (C) Venn diagram of genes supported or repressed by *RP11-424G14.1* knockdown in male keratinocytes and *XIST* knockdown in female keratinocytes compared to respective controls from microarray studies (FDR <0.05 for differentially expressed genes). (D) Expression levels of indicated genes upon scrambled (control) or *RP11-424G14.1* knockdown (KD) in male keratinocytes (top) and upon scrambled (control) or *XIST* knockdown (KD) in female keratinocytes (bottom) by qPCR. (E) Pathway enrichment analysis of microarray data showing the top 5 pathways impacted by the loss of *RP11-424G14.1* or *XIST*. FDR, false discovery rate. Mean ± stdev, *$p < 0.05$, Student's *t*-test.

As expected, as a lncRNA known to mediate X-chromosome inactivation in females, *XIST* RNA is expressed in human keratinocytes in a female-based manner (Figure 1A). The expression level of *XIST*, but not the control gene *GAPDH*, was reduced by its sequence-specific knockdown, suggesting specificity of expression detection (Figure S1). While *XIST* has been shown to decrease in expression in aged hematopoietic stem cells (HSCs),[23] we have found that in keratinocytes, the expression level of *XIST* did not associate with the age of donors (Figure 1A). Taken together, these data suggest tissue specificity in the expression change of *XIST* during aging. Based on these results, we focused our subsequent study on *RP11-424G14.1* in male keratinocytes and *XIST* in female keratinocytes.

3.2 | Genes and pathways regulated by sex-biased lncRNAs

Because little is known about the roles of *RP11-424G14.1* and *XIST* on a genome-wide level in human keratinocytes, we performed microarray to identify their downstream genes in an unbiased manner. We performed *RP11-424G14.1* and scrambled control knockdown in human male keratinocytes as well as *XIST* and scrambled control knockdown in human female keratinocytes (age- and race-matched), followed by microarray analyses. We identified four groups of differentially expressed genes (FDR <0.05), namely genes that were downregulated by *RP11-424G14.1* knockdown in male keratinocytes (i.e., *RP11-424G14.1*-supported), genes that were upregulated by *XIST* knockdown in female keratinocytes (i.e., *XIST*-repressed), genes that were upregulated by *RP11-424G14.1* knockdown in male keratinocytes (i.e., *RP11-424G14.1*-repressed), and genes that were downregulated by *XIST* knockdown in female keratinocytes (i.e., *XIST*-supported). Compared to *XIST*, *RP11-424G14.1* regulated a restricted set of genes, supporting the expression of 20 genes and repressing the expression of 4 genes (Figure 1C). Of the 20 *RP11-424G14.1*-supported genes in male keratinocytes, 4 genes (20%) were repressed by *XIST* in female keratinocytes, suggesting that these four genes are male-biased genes that are coregulated by the

TABLE 1 Known functions for co-targets of *RP11-424G14.1* and *XIST* as identified by microarray.

Gene	Known roles	Expression
IGFBP3	• Negative regulator of IGF signaling.[39] • Antagonist of TGF-βII receptor, arresting growth[34,38,40] • Facilitate NHEJ.[41] • Inhibit keratinocyte proliferation.[42] • Modulate carbohydrate and lipid metabolism.[43]	Male-biased
CXCL14	• Important for differentiation of dendritic cells into Langerhans cells.[44] • Immune surveillance through chemotaxis.[45] • Neutrophil, DC, and uterine NK cell chemoattractant.[46]	Male-biased
SEMA5A	• Inhibits synaptogenesis during neurogenesis.[47] • Promotes angiogenesis and inhibits apoptosis.[48]	Male-biased
WNK1	• Ion homeostasis in kidney and nervous system.[49] • Autophagy inhibition.[50] • Suppresses NLRP3 inflammasome activation, and therefore inflammation and pyroptosis.[51]	Male-biased
TSPAN1	• Promotes cell migration.[52] • Suppresses NFκB signaling.[53]	Female-biased
THBS2	• Inhibits angiogenesis.[54] • Promotes cell motility and migration.[55]	Female-biased

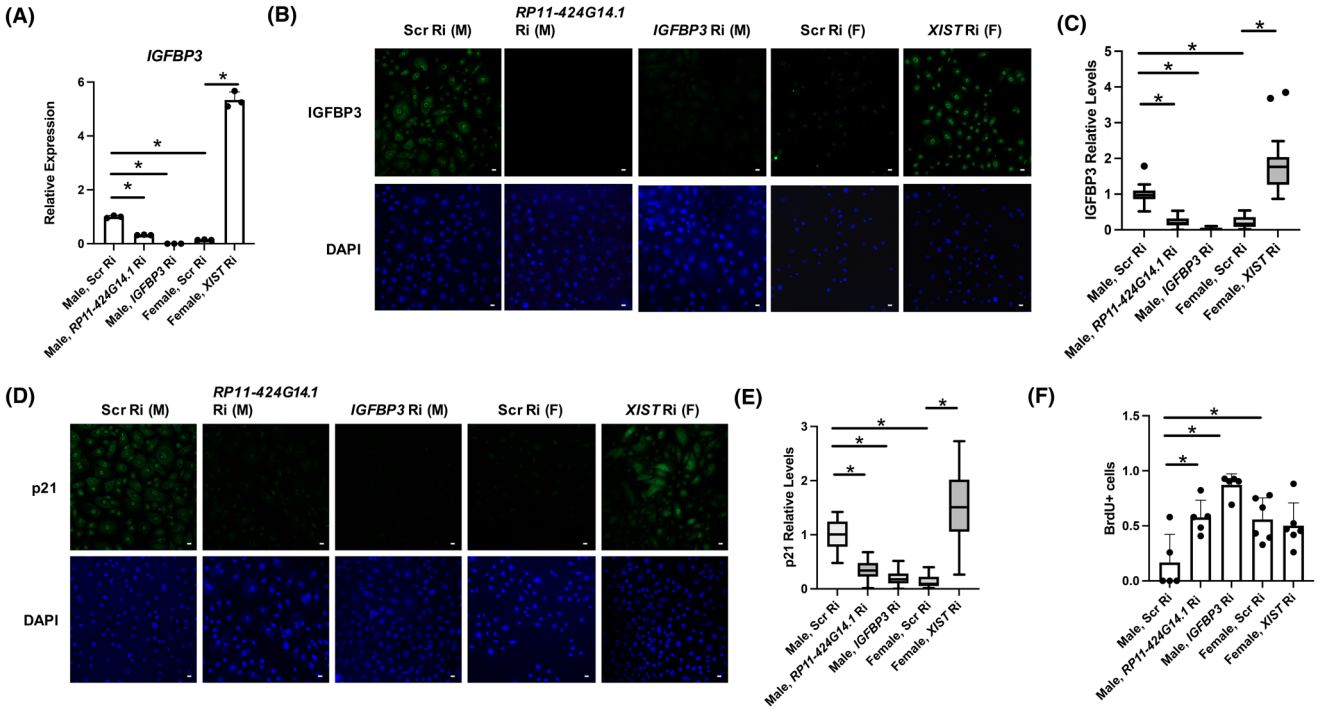

FIGURE 2 Regulation of senescence by male-biased molecules *RP11-424G14.1* and IGFBP3. (A) Expression level of *IGFBP3* in female or male keratinocytes by qPCR, with RNAi-mediated knockdown (Ri) of indicated genes. Scr, scrambled control. (B, C) Immunofluorescence staining (B) and quantification (C) of IGFBP3 with DAPI counterstain in male (M) or female (F) keratinocytes, with indicated knockdown or scrambled control. (D, E) Immunofluorescence staining (D) and quantification (E) of p21 with DAPI counterstain in male (M) or female (F) keratinocytes, with indicated knockdown or scrambled control. (F) Quantification of BrdU assay in male or female keratinocytes, with indicated knockdown or scrambled control. Average fluorescence intensity per cell is used in quantification. Scale bar, 10 μm. Mean ± stdev, $^*p < 0.05$, Student's *t*-test.

two lncRNAs (i.e. activated by *RP11-424G14.1* in males and repressed by *XIST* in females). These four genes are *IGFBP3*, *CXCL14*, *SEMA5A*, and *WNK1*. Of the 4 *RP11-424G14.1*-repressed genes in male keratinocytes, 2 genes (50%) were supported by *XIST* in female keratinocytes, suggesting that these two genes are female-biased genes that are coregulated by the two lncRNAs (i.e., repressed by *RP11-424G14.1* in males and activated by *XIST* in females). These two genes are *TSPAN1* and *THBS2*. We further validated the co-regulation of these six genes by *RP11-424G14.1* and *XIST* by independent qPCR experiments (Figure 1D) and summarized their known functions (Table 1).

Furthermore, gene ontology analyses suggested that the top pathways affected by *RP11-424G14.1* knockdown included the NF-κB pathway, consistent with known sex differences in inflammation. Intriguingly, both *RP11-424G14.1* and *XIST* knockdown significantly affected senescence-related pathways, in line with sexual dimorphism in aging (Figure 1E).

With available antibodies, we were able to confirm the regulation of *IGFBP3* (Insulin-like growth factor binding protein 3), among other potential leads, by *RP11-424G14.1* (Figure 2A,B, Figure S2). Because *IGFBP3* was the top candidate from the microarray experiment, we focused subsequent study on *IGFBP3* (insulin-like growth factor binding protein) on its potential role in cellular senescence, the co-regulated biological pathway.

3.3 | *IGFBP3* as a lncRNA-regulated male-biased gene as well as their modulation of senescence

As an *RP11-424G14.1*-supported and *XIST*-repressed gene from microarray results, we predicted that *IGFBP3* was a male-biased gene. Indeed, in independent experiments we confirmed the male-biased expression of IGFBP3 in human keratinocytes on the mRNA level and in subcellular compartments (Figure 2A,B). Additionally, we confirmed that in male keratinocytes, *RP11-424G14.1* knockdown reduced IGFBP3 observed within the cell (Figure 2A–C). On the contrary, in female keratinocytes, XIST knockdown increased IGFBP 3 observed within the cell (Figure 2A–C).

Using p21 as a marker for cell senescence,[24] we showed that age- and race-matched male keratinocytes expressed increased levels of p21 compared to female (Figure 2D,E),

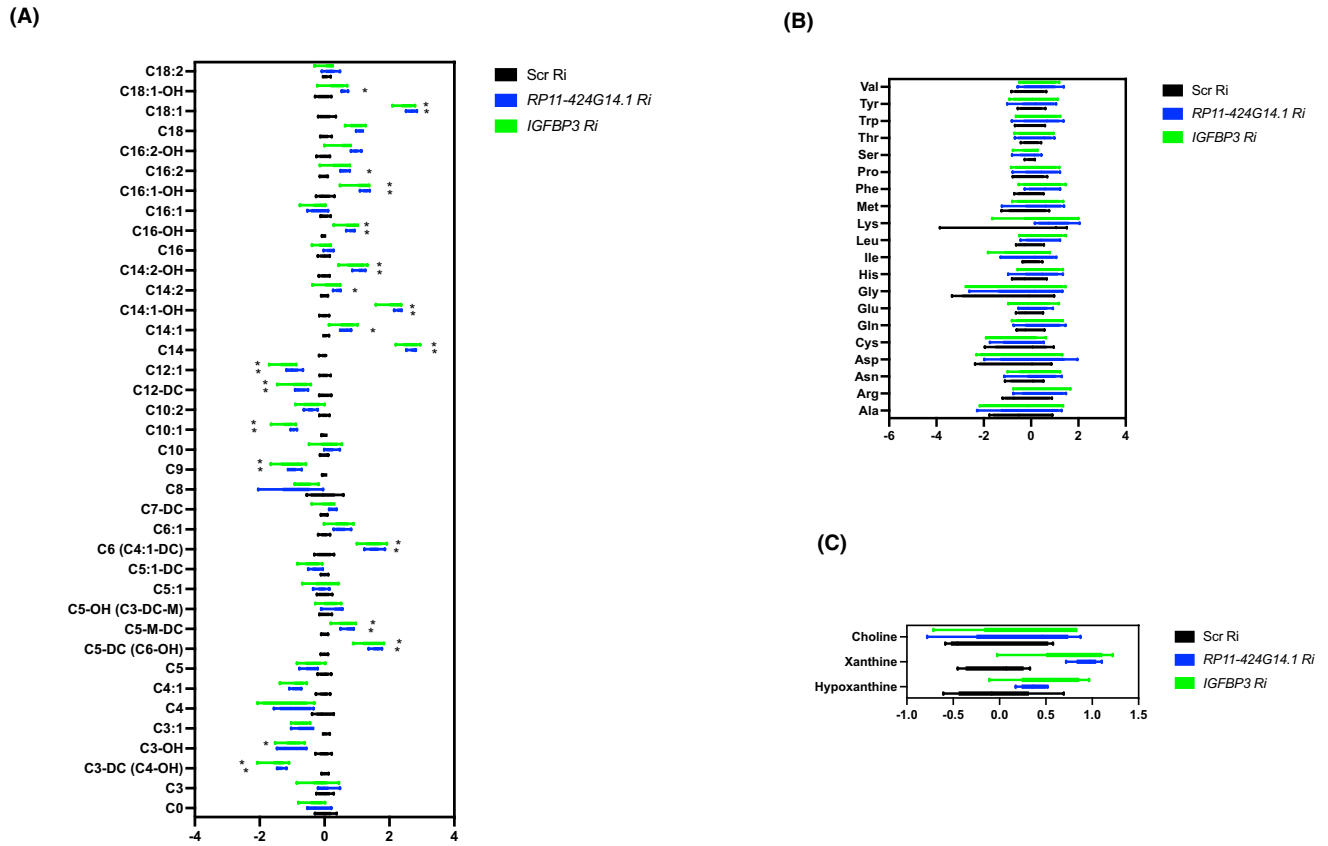

FIGURE 3 Regulation of metabolism by male-biased molecules *RP11-424G14.1* and IGFBP3. Metabolic profiling and quantification of acylcarnitine (A), amino acid (B) and nucleobases and vitamin and cofactors (C) in male keratinocytes with scrambled RNAi (scr Ri), *RP11-424G14.1*-specific RNAi (*RP11-424G14.1* Ri), and *IGFBP3*-specific RNAi (*IGFBP3* Ri). *, FDR <0.05.

consistent with previous reports that females exhibit lower biological ages as assessed by molecular markers.[4,5] Knockdown of *RP11-424G14.1* or *IGFBP3* reduced p21 levels in male keratinocytes, while knockdown of *XIST* increased p21 levels in female keratinocytes (Figure 2D,E). Similar results were obtained using the bromodeoxyuridine (BrdU) assay,[25] which showed limited proliferative capability of male keratinocytes compared to females (Figure 2F, Figure S3). The knockdown of *RP11-424G14.1* or *IGFBP3* restored proliferation of male keratinocytes (Figure 2F). Our results suggest that the *RP11-424G14.1* – IGFBP3 axis promotes senescence in male keratinocytes.

3.4 | Regulation of *IGFBP3* expression by insulin and nutrient levels

IGFBP3 has been historically used as a marker for growth hormone levels and is known to associate with high body mass index (BMI).[26,27] In addition to its role in binding IGF (insulin-like growth factor), IGFBP3 can signal through the IGF receptor, which can also relay signal from insulin, to regulate cell growth and survival.[27] Based on the known link of IGFBP3 to insulin signaling, we tested the hypothesis that IGFBP3 itself is regulated by insulin, a component of the keratinocyte growth media. Indeed, removal of insulin from the keratinocyte growth media reduced IGFBP3 levels, and similar effects were achieved by removing all growth supplements from the media (Figure S4). Removal of insulin or nutrients and the reduction in IGFBP3 were concurrent with the decrease in p21 (Figure S4). The decrease in p21 and cell number with insulin treatment is consistent with previous reports that increased insulin levels promote senescence of various human cell types and that nutrient restriction reduces senescence.[28,29] Insulin is known to signal through AKT signaling,[30] and in male keratinocytes we have found that AKT phosphorylation was dependent on *RP11-424G14.1* and IGFBP3, which suggests that IGFBP3 can both respond to and regulate insulin signaling (Figure S4).

Our ancestors have been dubbed "fat hunters", and to accommodate the large brains, the need for fat is thought to propel the evolution of *H. erectus*.[31] Given the upregulation of IGFBP3 by insulin and nutrient levels, we were curious to examine its expression regulation in other organisms. Intriguingly, we found that *RP11-424G14.1*, the promoter of IGFBP3 expression in human keratinocytes, was human-specific and not found in the rhesus monkey, mouse, or other lower organisms (Figure S5A). In the mouse skin, without *RP11-424G14.1*, there was no longer

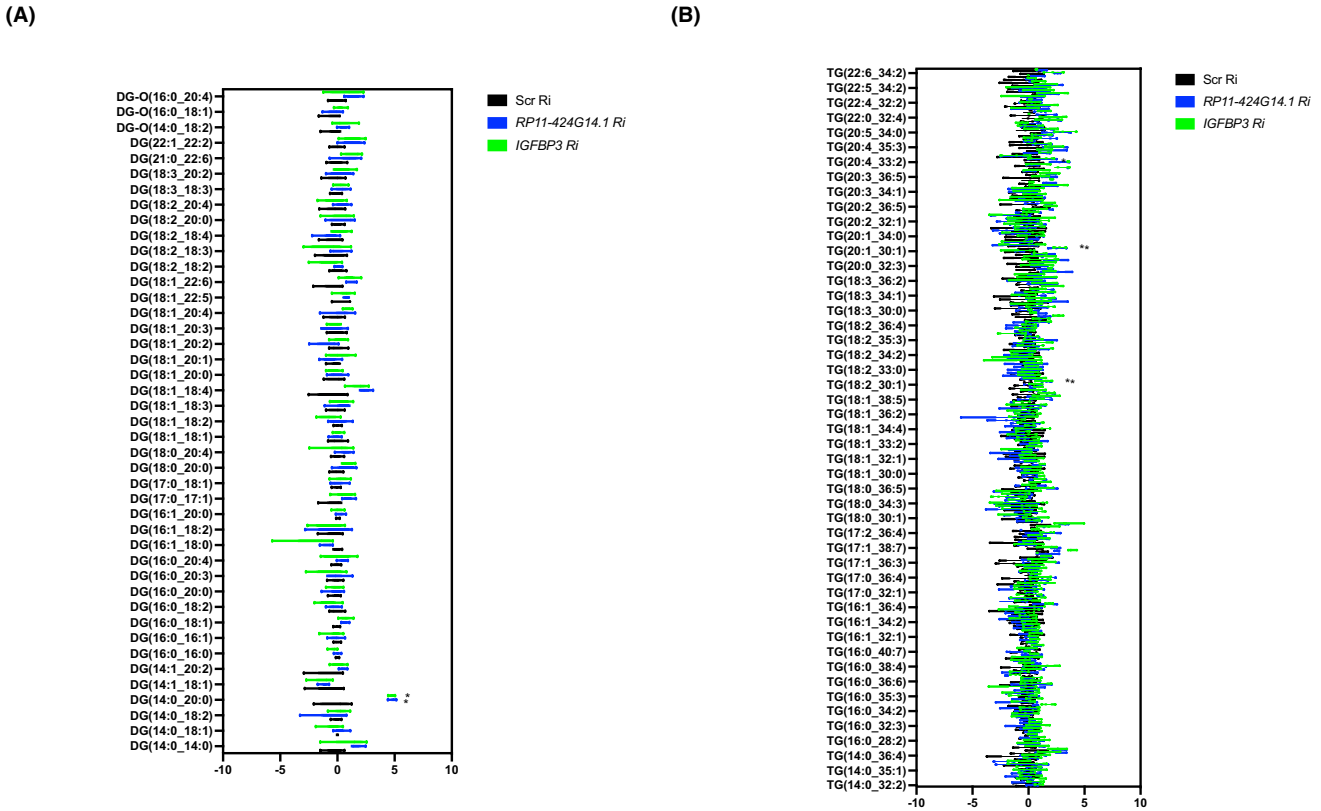

FIGURE 4 Regulation of diglycerides and triglycerides by male-biased molecules *RP11-424G14.1* and IGFBP3. Metabolic profiling and quantification of diglycerides (A) and triglycerides (B) in male keratinocytes with scrambled RNAi (scr Ri), *RP11-424G14.1*-specific RNAi (*RP11-424G14.1* Ri), and *IGFBP3*-specific RNAi (*IGFBP3* Ri). *, FDR <0.05.

a significant male bias in *Igfbp3* expression (Figure S5B). Therefore, the male-biased *RP11-424G14.1* – IGFBP3 axis is human-specific.

3.5 | Metabolism regulation by *RP11-424G14.1* and IGFBP3

Given the observed link between IGFBP3 and nutrition, we directly tested whether *RP11-424G14.1* and IGFBP3 regulated cell metabolism by metabolomic studies. Over 600 metabolites from 26 biochemical classes were profiled and quantified in keratinocytes with control or knockdown of *RP11-424G14.1* or IGFBP3. Consistent with a putative role of *RP11-424G14.1* – IGFBP3 in lipid metabolism, the most significant effect upon knockdown of *RP11-424G14.1* or IGFBP3 was observed in the acylcarnitine group. *RP11-424G14.1* or IGFBP3 knockdown resulted in increases in various long-chain acylcarnitines such as octadecenoylcarnitine, hydroxyhexadecenoylcarnitine, hydroxyhexadecanoylcarnitine, hydroxytetradecadienoylcarnitine, and tetradecanoylcarnitine, suggesting impairment of beta-oxidation of fatty acids (Figure 3A). In addition, *RP11-424G14.1* or IGFBP3 knockdown led to decreases in medium-chain acycarnitines including dodecenoylcarnitine, dodecanedioylcarnitine, decenoylcarnitine, and nonaylcarnitine (Figure 3A). Meanwhile, limited changes were detected for other lipid groups including diglycerides, triglycerides, phosphatidylcholines, lysophosphatidylcholines, or free or non-covalently bound fatty acids (Figures 4 and 5). Small molecules including nucleobases, amino acid, vitamins, and other metabolites remain unchanged upon the knockdowns (Figure 3C, Figure 5). The pattern of metabolite changes with knockdown in *RP11-424G14.1* and IGFBP3 is consistent with them working in the same pathway. Therefore, the male-biased factors *RP11-424G14.1* and IGFBP3 regulate lipid metabolism in keratinocytes.

3.6 | Regulation of NF-κB pathway by *RP11-424G14.1*

Enrichment of the NFκB pathway upon *RP11-424G14.1* knockdown from the microarray study prompted us to investigate the role of *RP11-424G14.1* in regulating

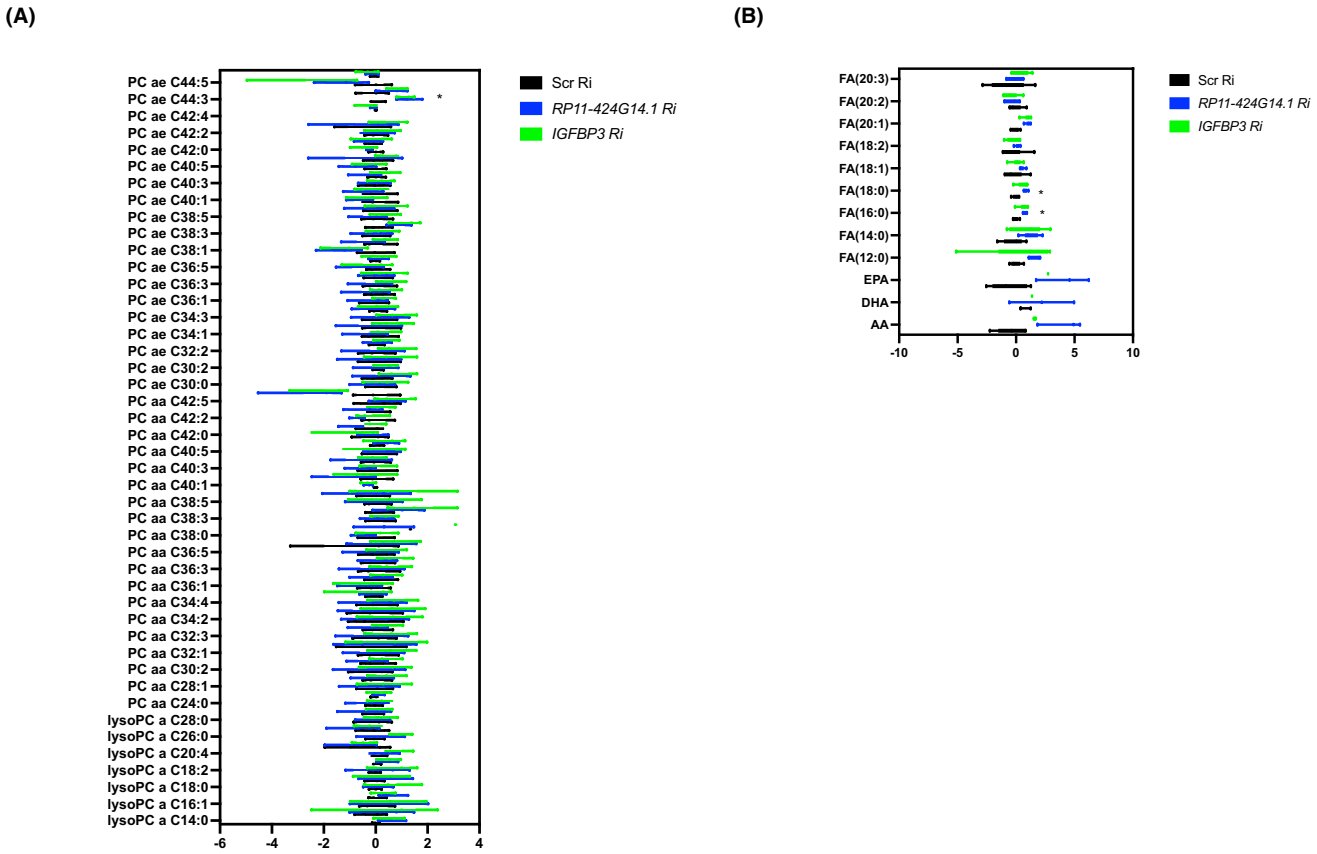

FIGURE 5 Regulation of phosphoglycerolipids and additional metabolites by male-biased molecules *RP11-424G14.1* and IGFBP3. Metabolic profiling and quantification of phosphoglycerolipids (A) and free fatty acids (B) in male keratinocytes with scrambled RNAi (scr Ri), *RP11-424G14.1*-specific RNAi (*RP11-424G14.1* Ri), and *IGFBP3*-specific RNAi (*IGFBP3* Ri). *, FDR <0.05.

inflammation, a female-biased response. During inflammation, activated NFκB translocates from cytosol to nucleus to coordinate with cofactors and regulate expression of inflammatory genes.[32] We simulated inflammation using TNFα, LPS, and IL-1β and found that the combination of *RP11-424G14.1* knockdown and TNFα stimulation promoted NFκB nuclear translocation and activation (Figure 6, Figure S6). Mechanistically, *RP11-424G14.1* inhibition of NFκB activation does not appear to function through canonical means, as TNFα induced IκBα degradation regardless of RP11 knockdown (Figure 6, Figures S6 and S7). Therefore, the lack of *RP11-424G14.1* in female keratinocytes potentiates TNFα-induced NFκB activation.

4 | DISCUSSION

With protein-coding genes making up only approximately 1 percent of the human genome, it is important to understand how the non-coding genetic elements may contribute to sexual dimorphism. Our study uncovers the multifaceted roles of a Y-linked noncoding RNA, *RP11-424G14.1*, in mediating sex differences in inflammation, senescence, and metabolism. We have found that in male keratinocytes, *RP11-424G14.1* signals through IGFBP3, which is regulated by *XIST* in an opposing manner in female cells. Through IGFBP3, we show that *RP11-424G14.1* and *XIST* modulate p21-mediated senescence in a manner consistent with the increased longevity of females.

The roles of the Y-linked *RP11-424G14.1* and X-linked *XIST* in regulation of inflammation, senescence, and metabolism suggest the contribution of sex chromosomes to these processes. While we have so far obtained no direct evidence for the regulation of *RP11-424G14.1* and *XIST* by sex hormones in cultured keratinocytes, future studies are warranted to test for a potential genetic and hormonal interaction. Indeed, studies show that *XIST* promotes insulin resistance in gestational diabetes,[33] suggesting a regulation of noncoding RNAs by dynamic changes in sex hormone levels.

RP11-424G14.1 is a human-specific lncRNA. Consistently, we have found that *RP11-424G14.1*-supported male bias of IGFBP3 is not conserved in the mouse. Intriguingly, the evolution of humans is tightly linked to the need to feed on and metabolize fat.[31] Our metabolomic data suggests that *RP11-424G14.1* and IGFBP3 work in a coordinated manner to regulate fatty acid metabolism, with a decrease in mobilized medium-chain acylcarnitines and increase in mobilized

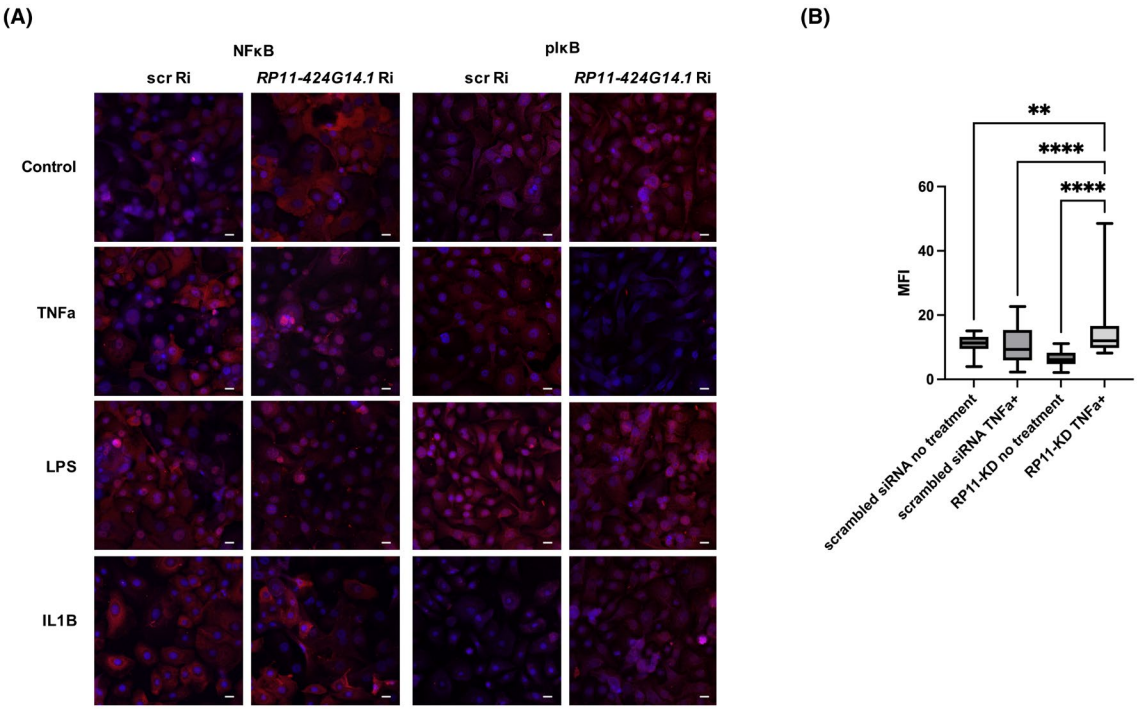

FIGURE 6 Regulation of NFκB nuclear translocation by *RP11-424G14.1*. (A) Immunofluorescence staining of NFκB and pIκBα in male keratinocytes with *RP11-424G14.1* knockdown (RP11-424G14.1 Ri) or scrambled knockdown (Scr Ri), with or without stimulation with TNFa, LPS, or IL1B. Scale bar, 10 μm. (B) Keratinocytes stained with DAPI to delineate their nuclei were analyzed for nuclear NFkB localization. Regions of interest (ROI) were drawn around nuclei using DAPI counterstain, and then anti-NFkB-GFP probe mean fluorescence intensity (MFI) within the ROI was measured with standardized laser intensity and exposure settings between conditions. Whisker-plot of nuclear NFκB signal calculated with mean fluorescence intensity (MFI) of $n \geq 199$. Kruskal–Wallis test was performed due to half-normal distribution and differences in group size ($\alpha = 0.05$). **, $P < 0.01$. ****, $P < 0.0001$.

long-chain acylcarnitines with their knockdown. The lack of change in diglycerides or triglycerides implies that the cells are not simply meeting the demands of increased proliferation on their membranes. Furthermore, *RP11-424G14.1* and IGFBP3 may regulate lipid metabolism through mitochondria homeostasis, as has been reported for IGFBP3.[34] Further study on the mechanism by which *RP11-424G14.1* and IGFBP3 regulate various classes of lipids is necessary to address these possibilities.

RP11-424G14.1 also has a role in protecting male keratinocytes from TNFα-induced inflammation. Knockdown of *RP11-424G14.1*, mimicking the female scenario, promotes the nuclear translocation of NFκB. Given the degradation of IκBα, we speculate that *RP11-424G14.1* functions on other regulatory components for NFκB subunits such as HDAC3 and IRFD themselves or the importins/exportins and modulates the nuclear translocation process.[35–37] lncRNAs are capable themselves of modifying proteins through ubiquitination or adenylation.[19] Otherwise, *RP11-424G14.1* is located on the Y chromosome upstream of KDM5D, a histone demethylase known for sexually dimorphic epigenetic regulation and interaction with HDACs.[38]

In conclusion, XIST and *RP11-424G14.1* are sex-chromosome-encoded lncRNAs that modulate sexual dimorphic pathways in females and males, respectively. While *XIST* has previously been described as an important factor in female bias towards autoimmunity, we have found that *RP11-424G14.1* plays an opposing role in males. In male keratinocytes, *RP11-424G14.1* promotes senescence, modulate fatty acid metabolism, and prevent TNFα-induced NFκB activation. Our data establishes RP11-424G14.1 as a Y-linked, human-specific lncRNA that plays a multifaceted role in regulating sexual dimorphism.

AUTHOR CONTRIBUTIONS

Conceptualization, K.K. and Y.L.; Methodology, K.K. and Y.L.; Data collection and analysis, K.K. and Y.L.; Writing, reviewing and editing, K.K. and Y.L. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon request.

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REFERENCES

1. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16:626-638.
2. Whitacre CC. Sex differences in autoimmune disease. *Nat Immunol*. 2001;2:777-780.
3. Manuel RSJ, Liang Y. Sexual dimorphism in immunometabolism and autoimmunity: impact on personalized medicine. *Autoimmun Rev*. 2021;20:102775.
4. Hagg S, Jylhava J. Sex differences in biological aging with a focus on human studies. *elife*. 2021;10:e63425.
5. Lemaitre JF, Ronget V, Tidiere M, et al. Sex differences in adult lifespan and aging rates of mortality across wild mammals. *Proc Natl Acad Sci USA*. 2020;117:8546-8553.
6. Mauvais-Jarvis F. Sex differences in energy metabolism: natural selection, mechanisms and consequences. *Nat Rev Nephrol*. 2024;20:56-69.
7. Varlamov O, Bethea CL, Roberts CT Jr. Sex-specific differences in lipid and glucose metabolism. *Front Endocrinol (Lausanne)*. 2014;5:241.
8. Taneja V. Sex Hormones Determine Immune Response. *Front Immunol*. 2018;9:1931.
9. Faulkner JL, Belin de Chantemele EJ. Sex hormones, aging and cardiometabolic syndrome. *Biol Sex Differ*. 2019;10:30.
10. Alemany M. Estrogens and the regulation of glucose metabolism. *World J Diabetes*. 2021;12:1622-1654.
11. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol*. 2008;8:737-744.
12. Link JC, Chen X, Arnold AP, Reue K. Metabolic impact of sex chromosomes. *Adipocytes*. 2013;2:74-79.
13. Sharma R, Harris VM, Cavett J, et al. Rare X chromosome abnormalities in systemic lupus erythematosus and Sjogren's syndrome. *Arthritis Rheumatol*. 2017;69:2187-2192.
14. Liang Y, Tsoi LC, Xing X, et al. A gene network regulated by the transcription factor VGLL3 as a promoter of sex-biased autoimmune diseases. *Nat Immunol*. 2017;18:152-160.
15. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489:57-74.
16. Kapranov P, Cheng J, Dike S, et al. RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science*. 2007;316:1484-1488.
17. Statello L, Guo CJ, Chen LL, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol*. 2021;22:96-118.
18. Zhang X, Wang W, Zhu W, et al. Mechanisms and functions of long non-coding RNAs at multiple regulatory levels. *Int J Mol Sci*. 2019;20:5573.

19. Schmitt AM, Garcia JT, Hung T, et al. An inducible long noncoding RNA amplifies DNA damage signaling. *Nat Genet*. 2016;48:1370-1376.
20. Loda A, Heard E. Xist RNA in action: past, present, and future. *PLoS Genet*. 2019;15:e1008333.
21. Bost C, Arleevskaya MI, Brooks WH, Plaza S, Guery JC, Renaudineau Y. Long non-coding RNA Xist contribution in systemic lupus erythematosus and rheumatoid arthritis. *J Clin Immunol*. 2022;236:108937.
22. Lovell CD, Anguera MC. Long noncoding RNAs that function in nutrition: Lnc-ing nutritional cues to metabolic pathways. *Annu Rev Nutr*. 2022;42:251-274.
23. Grigoryan A, Pospiech J, Kramer S, et al. Attrition of X chromosome inactivation in aged hematopoietic stem cells. *Stem Cell Reports*. 2021;16:708-716.
24. el-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. *Cell*. 1993;75:817-825.
25. Biran A, Zada L, Abou Karam P, et al. Quantitative identification of senescent cells in aging and disease. *Aging Cell*. 2017;16:661-671.
26. Horenz C, Vogel M, Wirkner K, et al. BMI and contraceptives affect new age-, sex-, and puberty-adjusted IGF-I and IGFBP-3 reference ranges across life span. *J Clin Endocrinol Metab*. 2022;107:e2991-e3002.
27. Varma Shrivastav S, Bhardwaj A, Pathak KA, Shrivastav A. Insulin-like growth factor binding Protein-3 (IGFBP-3): unraveling the role in mediating IGF-independent effects within the cell. *Front Cell Dev Biol*. 2020;8:286.
28. Kolb H, Kempf K, Martin S. Insulin and aging - a disappointing relationship. *Front Endocrinol (Lausanne)*. 2023;14:1261298.
29. Green CL, Lamming DW, Fontana L. Molecular mechanisms of dietary restriction promoting health and longevity. *Nat Rev Mol Cell Biol*. 2022;23:56-73.
30. Miao R, Fang X, Wei J, Wu H, Wang X, Tian J. Akt: A potential drug target for metabolic syndrome. *Front Physiol*. 2022;13:822333.
31. Ben-Dor M, Gopher A, Hershkovitz I, Barkai R. Man the fat hunter: the demise of Homo erectus and the emergence of a new hominin lineage in the middle Pleistocene (ca. 400 kyr) Levant. *PLoS One*. 2011;6:e28689.
32. Xiao C, Ghosh S. NF-κB, an evolutionarily conserved mediator of immune and inflammatory responses. *Adv Exp Med Biol*. 2005;560:41-45.
33. Xu Y, Kang X, Liu H, Jiang H, Wang W. LncRNA XIST promotes insulin resistance in gestational diabetes mellitus via the microRNA-181b-5p/NDRG2 axis. *Gen Physiol Biophys*. 2023;42:443-455.
34. Stuard WL, Titone R, Robertson DM. IGFBP-3 functions as a molecular switch that mediates mitochondrial and metabolic homeostasis. *FASEB J*. 2022;36:e22062.
35. Ruiz A, Palacios Y, Garcia I, Chavez-Galan L. Transmembrane TNF and its receptors TNFR1 and TNFR2 in mycobacterial infections. *Int J Mol Sci*. 2021;22:5461.
36. Leus NG, Zwinderman MR, Dekker FJ. Histone deacetylase 3 (HDAC 3) as emerging drug target in NF-κB-mediated inflammation. *Curr Opin Chem Biol*. 2016;33:160-168.
37. Tummers B, Goedemans R, Pelascini LP, et al. The interferon-related developmental regulator 1 is used by human papillomavirus to suppress NFκB activation. *Nat Commun*. 2015;6:6537.
38. Kuemmerle JF. IGFBP-3 activates TGF-β receptors and directly inhibits growth in human intestinal smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol*. 2004;287:G795-G802.
39. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions*. *Endocr Rev*. 1995;16:3-34.
40. Fanayan S, Firth SM, Baxter RC. Signaling through the Smad pathway by insulin-like growth factor-binding protein-3 in breast cancer cells. Relationship to transforming growth factor-beta 1 signaling. *J Biol Chem*. 2002;277:7255-7261.
41. Lin MZ, Marzec KA, Martin JL, Baxter RC. The role of insulin-like growth factor binding protein-3 in the breast cancer cell response to DNA-damaging agents. *Oncogene*. 2014;33:85-96.
42. Edmondson SR, Thumiger SP, Kaur P, et al. Insulin-like growth factor binding protein-3 (IGFBP-3) localizes to and modulates proliferative epidermal keratinocytes in vivo. *Br J Dermatol*. 2005;152:225-230.
43. Yamada PM, Mehta HH, Hwang D, Roos KP, Hevener AL, Lee KW. Evidence of a role for insulin-like growth factor binding protein (IGFBP)-3 in metabolic regulation. *Endocrinology*. 2010;151:5741-5750.
44. Schaerli P, Willimann K, Ebert LM, Walz A, Moser B. Cutaneous CXCL14 targets blood precursors to epidermal niches for Langerhans cell differentiation. *Immunity*. 2005;23:331-342.
45. Lu J, Chatterjee M, Schmid H, Beck S, Gawaz M. CXCL14 as an emerging immune and inflammatory modulator. *J Inflamm*. 2016;13:1.
46. Cao Q, Chen H, Deng Z, et al. Genetic deletion of Cxcl14 in mice alters uterine NK cells. *Biochem Biophys Res Commun*. 2013;435:664-670.
47. Carulli D, De Winter F, Verhaagen J. Semaphorins in adult nervous system plasticity and disease. *Front Synap Neurosci*. 2021;13:672891.
48. Sadanandam A, Rosenbaugh EG, Singh S, Varney M, Singh RK. Semaphorin 5A promotes angiogenesis by increasing endothelial cell proliferation, migration, and decreasing apoptosis. *Microvasc Res*. 2010;79:1-9.
49. Shekarabi M, Zhang J, Khanna AR, Ellison DH, Delpire E, Kahle KT. WNK kinase signaling in ion homeostasis and human disease. *Cell Metab*. 2017;25:285-299.
50. Gallolu Kankanamalage S, Lee AY, Wichaidit C, et al. WNK1 is an unexpected autophagy inhibitor. *Autophagy*. 2017;13:969-970.
51. Mayes-Hopfinger L, Enache A, Xie J, et al. Chloride sensing by WNK1 regulates NLRP3 inflammasome activation and pyroptosis. *Nat Commun*. 2021;12:4546.
52. Liu L, He B, Liu WM, Zhou D, Cox JV, Zhang XA. Tetraspanin CD151 promotes cell migration by regulating integrin trafficking. *J Biol Chem*. 2007;282:31631-31642.
53. Wang MD, Li HT, Peng LX, et al. TSPAN1 inhibits metastasis of nasopharyngeal carcinoma via suppressing NF-kB signaling. *Cancer Gene Ther*. 2024;31:454-463.
54. Bornstein P. Thrombospondins function as regulators of angiogenesis. *J Cell Commun Signal*. 2009;3:189-200.
55. Qi L, Sun B, Yang B, Lu S. CEBPB regulates the migration, invasion and EMT of breast cancer cells by inhibiting THBS2 expression and O-fucosylation. *Hum Mol Genet*. 2023;32:1850-1863.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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