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After skin wounding, noncoding dsRNA coordinates prostaglandins and Wnts to promote regeneration.

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Abbreviations: dsRNA (double-stranded RNA), NHEK (normal human epidermal keratinocyte),

PGE₂ (prostaglandin E₂), PWD (post-wounding day), TLR3 (toll-like receptor 3), WIHN (wound induced hair neogenesis)

Abstract

In a rare example akin to organogenesis in adult mammals, large wounds in mice lead to de novo morphogenesis of hair follicles. It is still not fully clear what controls this process, known as Wound Induced Hair Neogenesis (WIHN). In other tissues, prostaglandin E₂ (PGE₂) is an important effector of regeneration and has been shown to stimulate the Wnt/beta-catenin pathway, which in turn is known to control WIHN. Previously, our group has demonstrated that noncoding dsRNA released during wounding is both necessary and sufficient to stimulate WIHN through TLR3. Here, we hypothesize that dsRNA similarly induces the beta-catenin pathway through PGE₂. We find that WIHN levels correlate closely to Wnt7b production in vivo, and that dsRNA potently induces Wnt7b in a manner that requires prostaglandin-endoperoxide synthase 2 (Ptgs2). The Ptgs2 inhibitor celecoxib reduces dsRNA-induced WIHN and Wnt7b, and exogenous PGE₂ can rescue WIHN and Wnt7b. Although other wnts and pathways likely contribute, these results highlight noncoding dsRNA as an upstream coordinator of prostaglandin and Wnt levels in regeneration.

Introduction

In many animals, skin wounding results in the formation of fibrotic scar tissue, which lacks appendages such as hair follicles, sweat glands, and sebaceous glands. However, in rabbits (Breedis 1954) and mice (Ito et al, 2007), large full-thickness skin wounds are capable of completely regenerating hair follicles. Although these follicles arise from epidermal cells that reside outside the hair follicle stem cell niche, they are able to re-establish a hair follicle stem cell population and progress through all phases of the hair cycle (Ito et al, 2007). This process, known as wound-induced hair follicle neogenesis (WIHN), closely recapitulates the molecular

and morphological events of embryonic hair follicle development, and is one of the rare examples of complete organogenesis in adult mammals. The mechanism of WIHN is still not fully clear.

Recently, our group has identified double-stranded RNA (dsRNA) as a key upstream molecule linking damage sensing and skin regeneration. During wounding, noncoding dsRNA is released into the wound and initiates the regenerative WIHN response by binding to its receptor, TLR3, and activating downstream effectors such as IL-6. Although the origin of this noncoding dsRNA is not fully understood, we have hypothesized that it is released from dying or apoptotic epidermal cells in the wound bed. A similar mechanism has previously been characterized in UVB skin irradiation, whereby double-stranded noncoding U1 RNA is released from damaged keratinocytes and activates TLR3 (Bernard et al, 2012). The importance of dsRNA released from damaged cells is supported by our findings that increased epidermal damage correlates with higher TLR3 expression and regeneration, and that immune cells appear to be dispensable for this phenomenon (Nelson et al, 2015). Polyinosinic:polycytidylic acid (poly(I:C)), a dsRNA analogue and TLR3 receptor agonist, significantly increases the number of regenerated hair follicles when injected into healing wounds. Conversely, mice lacking TLR3 or exposed to dsRNase fail to regenerate hair follicles through WIHN (Nelson et al, 2015). TLR3 activation by dsRNA in keratinocytes can also induce gene expression and organelle formation characteristic of an epidermal barrier repair response (Borkowski et al, 2013).

Wnt signaling plays a critical role in stem cell regulation during development and regeneration in multiple tissue types, including the skin, blood, and intestines. In the canonical Wnt pathway,

Wnt ligands bind to Frizzled receptors and LRP5/6 coreceptors, inactivating the β -catenin destruction complex. This enables β -catenin to accumulate and subsequently translocate to the nucleus, where it participates in transcriptional regulation by interacting with Tcf/Lef transcription factors (Clevers et al, 2014). In the hair follicle, canonical Wnt- β -catenin signaling shifts the fate of stem cells from quiescence to proliferation and differentiation (Lien et al, 2014) (Choi et al, 2013), largely via the secretion of Wnt ligands from the skin (Myung et al, 2013). One particularly important ligand is Wnt7b, which regulates hair follicle stem cell homeostasis and hair follicle cycling. Epithelial ablation of Wnt7b disrupts hair follicle cycling and stem cell self-renewal (Kandyba and Kobiela 2014). Wnt7b is also expressed in healing skin after laser ablation (Zheng et al, 2014) and likely contributes to WIHN (Nelson et al, 2015), as Wnts and β -catenin are sufficient and necessary for WIHN (Ito et al, 2007).

Prostaglandins (PGs), which are bioactive lipids with diverse physiological effects in various tissues, are also released upon tissue damage. Prostaglandin-endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase 2 (COX-2), is a key upstream enzyme in the PG synthesis pathway that converts arachidonic acid to PGH_2 , which is further converted by downstream enzymes to other PGs such as PGD_2 and PGE_2 . In skin wounds, PGD_2 and PGE_2 are reciprocally expressed during healing, with PGD_2 levels being associated with low WIHN regeneration (Nelson et al, 2013). In contrast with the anti-regenerative effects of PGD_2 , PGE_2 promotes regeneration in multiple tissue types, including the blood, colon, and liver (Zhang et al, 2015). During wound healing in the skin, PGE_2 is produced in response to apoptotic cell death through the caspase-dependent "phoenix rising" pathway (Li et al, 2010).

While there is known crosstalk between Wnt and PG signaling in regeneration (Goessling et al, 2009), the upstream coordination and physiologic contexts which might connect Wnt and PG signaling are not clear. Here, we investigate the hypothesis that noncoding dsRNA and TLR3-mediated damage-sensing stimulate a PG-dependent mechanism to activate Wnt7b secretion from epidermal keratinocytes and induce WIHN.

Results

dsRNA induces regeneration and Wnt7b expression via the TLR3 / IL-6 / STAT3 axis

In our previous study (Nelson et al, 2015), we showed that whole hair follicle regeneration in wounds can be induced by polyinosinic:polycytidylic acid (poly(I:C)), a synthetic dsRNA (Fig. 1A). Since it has been shown that the Wnt/ β -catenin pathway is necessary and Wnt7a is sufficient for WIHN (Ito et al, 2007), we first looked for potential Wnt ligands which might be induced by dsRNA. We performed gene expression microarrays of vehicle- or dsRNA-treated cultured normal human epidermal keratinocytes (NHEKs). Among all the Wnt ligands that were screened, Wnt7b was the most strongly induced transcript (Fig. 1B). Given the necessary role of Wnt7b in hair follicle biology (Kandyba and Kobiela 2014), we therefore focused on this gene as an induced target of TLR3 pathway activation during WIHN. We next wondered whether poly(I:C) would stimulate in vivo expression of Wnt7b, which regulates hair follicle morphogenesis. Immunofluorescent staining revealed that Wnt7b protein is produced in the basal layer epidermis of healing mouse skin wounds injected with poly(I:C) (Fig. 1C). Additionally, in cultured NHEKs, poly(I:C) upregulates WNT7B mRNA transcript levels in a dose-dependent manner (Fig. 1D), as well as Wnt7b protein (Fig. 1E).

We previously reported (Nelson et al, 2015) that dsRNA stimulates WIHN by binding to its receptor, TLR3, and activating the IL-6 / STAT3 signaling pathway. Consistent with these findings, mice lacking TLR3, which have almost no WIHN (Nelson et al, 2015), display a marked reduction of WNT7B mRNA in the wound during healing (Fig. 2A). Furthermore, IL-6 rescues WIHN in TLR3 knockout mice (Nelson et al, 2015) and also rescues WNT7B levels (Fig. 2B). Likewise, mice lacking STAT3 in the epidermis display both reduced WIHN (Nelson et al, 2015) and reduced WNT7B expression (Fig. 2C). In cultured NHEKs, siRNA silencing of TLR3 abrogates the induction of WNT7B by poly(I:C) (Fig. 2D). Taken together, these results indicate that Wnt7b is expressed in healing skin wounds in response to dsRNA, and correlates closely with activation of the TLR3 / IL-6 / STAT3 pathway and WIHN. Thus, Wnt7b levels are a robust proxy for WIHN, though it is likely other Wnts contribute to WIHN.

Prostaglandins are required for Wnt7b expression and hair follicle regeneration

Next, we investigated the role of prostaglandins in the wound healing response to dsRNA. The mRNA transcript level of the inducible prostaglandin synthase PTGS2 is upregulated in mouse skin wounds up to 5 days after wounding (Fig. 3A). Similarly, poly(I:C) upregulates PTGS2 mRNA in NHEKs in a dose-dependent manner, again underscoring the fidelity for NHEKs as a model (Fig. 3B). Consistent with the reciprocal effects of PGD₂ and PGE₂ on regeneration, the mRNA levels of prostaglandin D₂ synthase (PTGDS) and prostaglandin E₂ synthase (PTGES) were downregulated and upregulated respectively by poly(I:C) (Fig. 3C). In the same gene expression microarray of vehicle- versus dsRNA-treated NHEKs depicted in Fig. 1B, PTGS2 was among the most upregulated genes; interestingly, PTGS1, a constitutively active prostaglandin synthase, was strongly downregulated. Additionally, 15-hydroxyprostaglandin

dehydrogenase (HPGD), an enzyme that degrades prostaglandins and inhibits regeneration, was also strongly downregulated (Fig. 3D).

Importantly, siRNA silencing of PTGS2 (Fig. 4A) abrogates the induction of WNT7B by poly(I:C) (Fig. 4B). We wondered whether the exogenous addition of prostaglandin E₂ (PGE₂), which has previously been shown to have pro-regeneration effects, would rescue WNT7B expression. Interestingly, while PGE₂ did not rescue WNT7B mRNA, it did rescue Wnt7b protein (Fig. 4C, D). In contrast, addition of PGD₂ resulted in significant cytotoxicity.

Finally, to corroborate our in vitro results, we examined the effects of prostaglandins on wound-induced hair follicle regeneration in vivo. Inhibition of prostaglandin synthesis by celecoxib, a selective PTGS2 inhibitor, significantly reduced the number of regenerated hair follicles (Fig. 4E, F), while addition of exogenous PGE₂ rescued the regenerative phenotype (Fig. 4G, H). Wnt7b on immunofluorescence staining of wound edges at PWD4 appeared to match these regeneration levels (Supplemental Figure 1). It is important to note that whereas in our previous study (Fig. 1A) we used an inherently high-regenerating B6/FVB/SJL mixed mouse strain, in the present study we have used an inbred C57BL6/NJ strain with a lower baseline level of regeneration. This was in order to demonstrate that lower WIHN levels can be rescued through robust activation by poly(I:C), inhibition by celecoxib, and rescue by PGE₂. Taken together, these results indicate that noncoding dsRNA coordinates the synthesis of prostaglandins, particularly PGE₂, to promote Wnt7b expression and hair follicle regeneration during wound healing.

Discussion

In this study, we show that noncoding dsRNA coordinates prostaglandin and Wnt signaling in regeneration. Poly(I:C), a synthetic dsRNA, activates Wnt7b in wounds and results in de novo hair follicle regeneration. While there is some strain-, litter-, and operator- dependent variation in the absolute number of hair follicles regenerated during WIHN, poly(I:C) consistently increases hair follicle regeneration in strain-matched and litter-matched experiments across multiple mouse strains, both here and in our previously published work (Nelson et al, 2015). We chose Wnt7b as a readout for pro-regenerative Wnt signaling during WIHN because of the fact that WIHN closely recapitulates the events of embryonic hair follicle development (Ito et al, 2007), where Wnt7b has been shown to play an early role in regulating hair follicle morphogenesis (Kandyba and Kobiela 2014). Our results show that Wnt7b expression in wounds correlates closely with the regenerative WIHN outcomes we previously observed, suggesting that Wnt7b regulates hair follicle morphogenesis during WIHN analogously to its role in embryogenesis. However, it is likely that other wnts and pathways contribute to WIHN as well.

Furthermore, we show that dsRNA-induced Wnt7b expression and WIHN outcomes are prostaglandin-dependent. Inhibiting prostaglandin synthesis using celecoxib or PTGS2-specific siRNA dramatically reduces Wnt7b production in keratinocytes as well as functional WIHN regeneration in mice, and both effects can be rescued by the addition of PGE₂. Interestingly, in cultured keratinocytes, PGE₂ rescues Wnt7b protein but not mRNA levels, suggesting that PGE₂ may affect the protein stability or mRNA translation efficiency of Wnt7b rather than regulating it at the transcriptional level. Further mechanistic studies are necessary to characterize the effects of PGE₂ on Wnt7b protein expression and stability.

PGE₂ was discovered to be important in regeneration through a blind screen of compounds which activated zebrafish hematopoietic stem cells (North et al, 2007). Subsequent research has proven that PGE₂ promotes regeneration by interacting with the β -catenin/Wnt pathway through a detailed mechanism involving cAMP/PKA signaling (Goessling et al, 2009). Additionally, it has been shown that PGE₂ is one of the primary prostaglandins produced in the skin in response to cell damage (Li et al, 2010), consistent with our result that adding PGE₂ back to the skin rescues regeneration after celecoxib treatment. However, the upstream regulators that coordinate stem cell function and regeneration to promote PGE₂ production have not been elucidated. Our work is significant because we demonstrate that noncoding dsRNA and innate immunity pathways such as TLR3 are one example of upstream coordination of PGE₂ production to activate the Wnt pathway. Future work should examine if the same pathway is important in other models of regeneration.

An important question raised by this work is whether aspirin would be predicted to alter regeneration and Wnt levels in healing skin. Aspirin, a nonsteroidal anti-inflammatory drug (NSAID) commonly used as an analgesic, decreases prostaglandin production by blocking the activity of cyclooxygenases. Previous studies have shown that aspirin delays re-epithelialization in the skin by inhibiting the cyclooxygenase-dependent production of 12-hydroxyheptadecatrienoic acid (12-HHT), a key lipid mediator in wound healing (Liu et al, 2014). Our work suggests an alternative, prostaglandin-dependent mechanism to explain the adverse effect of aspirin on wound healing.

Recently, inhibitors of 15-hydroxyprostaglandin dehydrogenase (HPGD, also known as 15-PGDH) have been proposed to enhance regeneration (Zhang et al, 2015). HPGD is an enzyme that degrades prostaglandins, and has been shown in published microarray data (Karim et al, 2011) to be dramatically inhibited by dsRNA in keratinocytes. This downregulation of HPGD by dsRNA, together with the marked upregulation of PTGS2 that we observed, is consistent with the ability of noncoding dsRNA to enhance regeneration by modulating prostaglandin levels.

Previously, our group found that prostaglandins E₂ and D₂ are elevated early and late, respectively, following wounding. Furthermore, we found that prostaglandin D₂ inhibits wound-induced hair neogenesis (Nelson et al, 2013). In the present study, we show that prostaglandin E₂ enhances WIHN. This is consistent with our model that PGD₂ and PGE₂ have opposing effects on hair follicle regeneration, and points to a potential regulatory mechanism where PGE₂ provides a pro-regenerative signal during the early stages of wound healing to initiate WIHN, and PGD₂ is produced later to conclude regeneration. Future studies could examine the negative feedback relationship between these two molecules, consistent with the role of PGD₂ in inhibiting hair growth during male pattern hair loss (Garza et al, 2012).

Since non-prostaglandin pathways are likely highly relevant to WIHN, future studies should also assess other pathways known to modulate Wnt levels. One important example is the BMP pathway, where Noggin is known to inhibit BMP and promote Wnt activity and hair follicle function (Botchkarev et al, 1999) (Botchkarev et al, 2001). Although Noggin would be predicted to promote WIHN it has not yet been tested. Finally, investigating the equivalent WIHN biology in other animal systems would also be highly productive (Hughes et al, 2011).

In summary, we have identified a previously unidentified mechanism for noncoding dsRNA to stimulate de novo hair follicle regeneration in wounds. Further mechanistic study and testing of dsRNA in human subjects is important for developing new therapies for regeneration. Also, since dsRNA is likely only one of a variety of pathways which stimulate regeneration, further study of WIHN is important.

Materials and Methods

WIHN assay

The wound-induced hair neogenesis (WIHN) assay was performed in C57BL6/NJ mice as previously described (Nelson et al, 2015). Briefly, 1 cm² full-thickness wounds were made on the backs of 21-day-old male and female mice. On post-wounding day 3 (PWD3), 100 µg/ml high molecular weight polyinosinic-polycytidylic acid (poly(I:C)) (Invivogen, San Diego, CA) was injected into the healing wounds. Prostaglandin signaling was inhibited by co-administering 20 µg/ml celecoxib (Sigma-Aldrich, St Louis, MO) on PWD3. Similarly, rescue of PG signaling was accomplished by co-administering 50 µM 16,16-dimethyl prostaglandin E₂ (dmPGE₂) (Cayman Chemical, Ann Arbor, MI). Regenerated hair follicles were quantified using confocal scanning light microscopy on PWD21-24 using a Vivascope 1500. All images shown are representative of multiple biological replicates. Strain and vehicle matched animals were used for all comparisons. All animal protocols were approved by the Johns Hopkins University Animal Care and Use Committee.

Analysis of gene expression by qRT-PCR in knockout mice was performed as previously described (Nelson et al, 2015). For comparison of wild-type and TLR3 knockout mice, B6;129SF2/J and B6;129S1-Tlr3^{tm1Flv/J} mice were used. For IL-6 rescue experiments, B6N.129S1-Tlr3^{tm1Flv/J} mice were injected with vehicle or 500 ng rmIL-6 (R&D Systems). For comparison of wild-type and STAT3 knockout mice, K5-ERT2-Cre x Stat3^{fl/fl} mice were injected with tamoxifen intraperitoneally every other day from PWD0-PWD14 to ablate the Stat3 gene. Wounded tissue was collected at WD4-12 for analysis.

Cell culture

Epidermal keratinocytes were isolated from neonatal foreskins and passaged up to two times before use. Cells were plated on collagen-coated dishes and treated with 10 µg/ml poly(I:C) or 10 µM dmPGE₂ for 4 days in KGM-GOLD keratinocyte media (Lonza, Walkersville, MD), which was replenished every other day. Transfection was performed with the Lipofectamine 2000 reagent (ThermoFisher, Waltham, MA) according to the manufacturer's protocol, using either untargeted or PTGS2 siRNA (Sigma-Aldrich). All data is representative of at least three independent repeats.

qRT-PCR

Total RNA was isolated from cells using the SpinSmart RNA kit (Denville Scientific, Holliston, MA) with on-column digestion of contaminating gDNA by DNase I (Qiagen, Valencia, CA), then converted to cDNA using the High Capacity cDNA Reverse Transcription Kit (ThermoFisher). qRT-PCR analysis was performed using inventoried TaqMan probes

(ThermoFisher) and Taqman Fast Advanced Master Mix (ThermoFisher). Fold changes were calculated using the comparative $\Delta\Delta\text{CT}$ method by normalizing to RPLP0.

Full-thickness skin samples were harvested from mice at around PWD11-12, on the day that the scabs detached from the wounds, and stored in RNALater (Sigma, St Louis MO) prior to processing. Skin samples were trimmed to remove tissue surrounding the re-epithelialized scar, homogenized using a tissue grinder, processed with RNeasy Fibrous Tissue kit (Qiagen), and converted to cDNA using the High Capacity cDNA Reverse Transcription kit (ThermoFisher). qRT-PCR was performed using inventoried TaqMan probes, and fold changes were calculated using the comparative $\Delta\Delta\text{CT}$ method by normalizing to β -actin.

Gene expression microarray analysis

NHEKs were treated with vehicle or 30 $\mu\text{g/ml}$ poly(I:C) for 24 hours, followed by 72 hours in KGM-GOLD media alone. RNA was isolated using the RNeasy kit (Qiagen) according to the manufacturer's instructions, and submitted to the JHMI Deep Sequencing and Microarray core facility for microarray analysis using the Affymetrix GeneChip PrimeView Human Gene Expression array. These analyses have been submitted to the GEO database (GSE92646).

Immunoblotting

To enable detection of secreted proteins, GolgiStop Protein Transport Inhibitor (BD Biosciences, Franklin Lakes, NJ) was added to the culture medium for 6 hours prior to sample collection. Whole cell extracts were prepared using M-PER Mammalian Protein Extraction Reagent

(ThermoFisher) containing Halt Protease Inhibitor Cocktail (ThermoFisher). Proteins were separated under denaturing conditions on a NuPage 4-12% Bis-Tris gel (ThermoFisher), transferred onto a PVDF membrane, and probed overnight with rabbit polyclonal anti-Wnt7b antibody (1:1000, ab94915, Abcam, Cambridge, MA) followed by an HRP-conjugated secondary antibody. Bands were detected on a digital imaging system using SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher) and quantified by normalizing to β -actin. All blots shown are representative of at least three independent repeats.

Immunohistochemistry

Frozen tissue sections were fixed in 4% paraformaldehyde for 10 minutes, blocked in 5% fetal bovine serum, then probed overnight with rabbit anti-Wnt7b (1:200, Abcam) followed by an Alexa Fluor 488-conjugated secondary antibody (ThermoFisher).

Conflict of Interest

The authors state no conflict of interest.

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Author Contributions

ASZ, AL, TSR and LAG designed the studies, analyzed and interpreted the results. ASZ, AL, and LAG co-wrote the paper. In addition, MM assisted with laboratory experiments. All members discussed results and help formulate ongoing conduct of the project.

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Figure Legends

Fig. 1. Poly(I:C) induces WNT7B expression in wounds and cultured NHEKs. (A)

Representative confocal scanning light microscopy (CSLM) image of regenerated hair follicles in C57BL6/NJ mouse wounds injected with or without polyinosinic:polycytidylic acid (poly(I:C)). (B) Wnt ligands most strongly upregulated or downregulated in gene expression microarray comparing NHEKs treated with vehicle or poly(I:C), published under (GSE92646) (C) Representative immunofluorescent image of Wnt7b protein in C57BL6/NJ mouse wounds (scale bar = 100 μ m). (D) qRT-PCR analysis of WNT7B mRNA expression in NHEKs (n=3) treated with various doses of poly(I:C). (E) Western blot analysis of Wnt7b protein expression in NHEKs treated with or without poly(I:C), with β -actin as a loading control.

Fig. 2. WNT7B expression is regulated through the TLR3/IL-6/STAT3 axis. (A) qRT-PCR analysis of WNT7B mRNA expression in background (C57BL6/NJ) and TLR3 KO mouse wounds (n=8). (B) WNT7B mRNA expression in TLR3 KO mouse wounds injected with vehicle or recombinant IL-6 (n=8). (C) WNT7B mRNA expression in background or STAT3 KO mouse wounds (n=8). (D) WNT7B mRNA expression in NHEKs transfected with mock or TLR3-specific siRNA, followed by treatment with or without poly(I:C). *denotes $p < 0.05$.

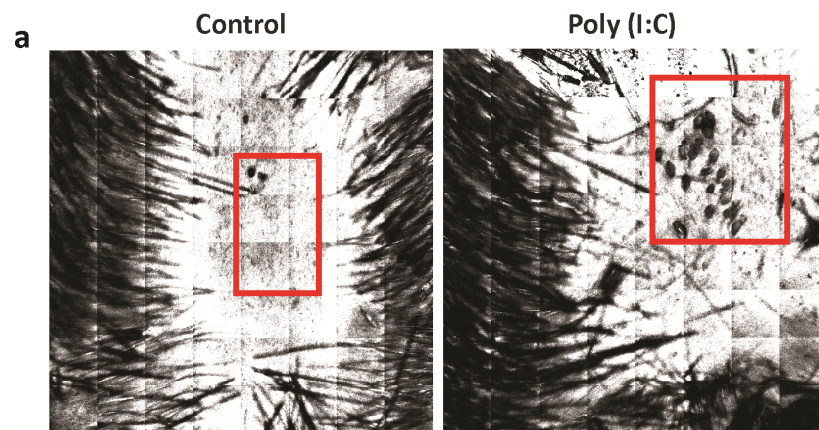
Fig. 3. Poly(I:C) induces PTGS2 expression in wounds and cultured NHEKs. (A) qRT-PCR analysis of PTGS2 mRNA expression in mouse wounds at post-wound day (PWD) 1, 3, and 5, normalized to pre-wound levels. (B) PTGS2 mRNA expression in NHEKs treated with various doses of poly(I:C). *denotes $p < 0.05$. (C) PTGDS and PTGES mRNA expression in NHEKs treated with poly(I:C). (D) Prostaglandin synthases 1 and 2 (PTGS1/2) and 15-hydroxyprostaglandin dehydrogenase (HPGD) in gene expression microarray in Fig. 1B.

Fig. 4. PTGS2 is required for poly(I:C)-induced WNT7B expression. (A) PTGS2 knockdown efficiency in NHEKs transfected with mock or PTGS2-specific siRNA. (B) qRT-PCR analysis of WNT7B mRNA expression in NHEKs (n=3) transfected with mock or PTGS2-specific siRNA, followed by treatment with or without poly(I:C), with the addition of prostaglandin E₂ (PGE₂), prostaglandin F₂ α (PGF₂ α), or 11 β -prostaglandin F₂ α (11b-PGF₂ α). ‡Prostaglandin D₂ induced significant cytotoxicity. (C) Representative western blot of Wnt7b protein expression in NHEKs treated as in Fig. 4B (n=3). (D) Western blot quantification for n=3 repeats performed as described in Fig. 4C. (E) Representative confocal scanning light microscopy (CSLM) image of

regenerated hair follicles in mouse wounds injected with poly(I:C), with or without celecoxib. (F) Quantification of hair follicles in CSLM images from Fig. 4D (n=15-17). (G) Representative confocal scanning light microscopy (CSLM) image of regenerated hair follicles in mouse wounds injected with poly(I:C) and celecoxib, with or without dmPGE₂. (H) Quantification of hair follicles in CSLM images from Fig. 4E (n=10-11). *denotes p<0.05.

Supplementary Figure 1 WNT7B expression during WIHN rescue with PGE₂. Wnt7b staining of WD4 wound beds of mice treated as in Figure 4E-H.

Supplementary Figure 2 High correlation between mouse in vivo wounding regeneration response and human in vitro dsRNA response. GEO database (GSE92646) was ranked according to genes most upregulated by Poly (I:C) in NHEKs. GEO database (GSE50418) was ranked according to genes most upregulated in wounded high regenerating mice versus low regenerating mice. The top 26 concordant and discordant genes are listed in the table showing the strength of the overlap between these two models-- including PTGS2.



b

| Rank | Gene | Fold Change |
|-------|--------|-------------|
| 187 | WNT7B | 5.09285 |
| 327 | WNT7B | 3.74295 |
| 961 | WNT5B | 2.22854 |
| 1391 | WNT7A | 1.94719 |
| 1549 | WNT3 | 1.87652 |
| 3428 | WNT10A | 1.51208 |
| 3799 | WNT7B | 1.47573 |
| 3801 | WNT7B | 1.47573 |
| 5700 | WNT5A | 1.3422 |
| 6311 | WNT5A | 1.31089 |
| 43144 | WNT16 | -1.31241 |
| 43414 | WNT3A | -1.32188 |
| 44314 | WNT10B | -1.36124 |
| 46348 | WNT2B | -1.49845 |

