Animal Model

Effects of in Vivo Heregulin β1 Treatment in Wild-Type and ErbB Gene-Targeted Mice Depend on Receptor Levels and Pregnancy

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Mice heterozygous (+/-) for either heregulin (HRG), ErbB2, or ErbB3 were created by gene targeting, resulting in the loss of one functional gene copy and an associated decrease in targeted protein. We examined the in vivo activity of recombinant HRG peptide, rHRG β1 (amino acids 177 to 241), in the three heterozygous mouse lines and in wild-type (WT) mice, both pregnant and nonpregnant. Nonpregnant WT and HRG(+/-) mice of both sexes were sensitive to rHRG β1 treatment as evidenced by a high mortality rate associated with abdominal enlargement and parietal cell loss. However, pregnant WT mice and ErbB2 and ErbB3 heterozygous mice treated with rHRG β1 were less affected, with significantly lower mortality rates and a less severe abdominal phenotype. Histological analysis revealed extensive breast ductal hyperplasia in females of all genotypes after rHRG β1 treatment. Hyperplasia of other epithelial tissues such as the pancreas and intestine and the growth of cardiac nerve bundles were also observed, independent of sex. (Am J Pathol 2001, 158:1871–1880)

Amplification and overexpression of ErbB2 (HER2), a type-1 receptor tyrosine kinase of the epidermal growth factor receptor (EGFR) (ErbB) family, is found in ~30% of human breast cancers and is associated with poor prognosis.1,2 Overexpression of ErbB2 is thought to lead to ligand-independent ErbB2:ErbB2 dimer formation, resulting in constitutive activation and signaling through the potent ErbB2 tyrosine kinase pathway.3 ErbB2 overexpression is also implicated in other human cancers of epithelial origin such as ovarian, gastric, pancreatic, and non-small cell lung cancer.4

The heregulins (HRGs), a family of structurally diverse glycoproteins with at least 15 spliced isoforms, indirectly activate ErbB2 signaling by binding to co-receptors (ErbB3, ErbB4) that can in turn dimerize with ErbB2 allowing HRG-induced signaling to proceed by receptor tyrosine phosphorylation.5–7 Interestingly, ErbB4 is an active tyrosine kinase receptor,7–10 whereas ErbB38,11 is catalytically impaired.12,13 Therefore, although tyrosine kinase activation of both ErbB2 and ErbB4 can occur in the ErbB2:ErbB4 heterodimer, only ErbB2 tyrosine kinase activation is possible in the ErbB2:ErbB3 heterodimer. ErbB3, however, preferentially signals through the SH2-containing PI-3-kinase pathway.14–17 Thus, the heterodimers ErbB2:ErbB3 and ErbB2:ErbB4 are the preferred HRG signaling complexes.18–20

The absence of either the entire HRG ligand family or the ErbB2, ErbB3, or ErbB4 receptor in gene-targeted mice resulted in embryonic lethality because of cardiac defects.21–25 The ErbB3 null mutant illustrated additional roles of HRG in cerebellar development, in the development of neural crest-derived lineages, and in epithelial-mesenchymal interactions critical for organogenesis.23

Given the pleiotropic activity that is attributed to the HRG family, it was of interest to determine the dominant biological effects observed in mice with different ErbB and HRG background when treated with rHRG β1. Therefore, heterozygous (+/-) mice, with only one functional copy of their targeted gene, were created for HRG, ErbB2, and ErbB3 by homologous recombination. These mice had decreased levels of their targeted gene product compared to wild-type (WT) mice,23,26 establishing a unique model to study the interactions of HRG and its receptors, with the expectation that reduced levels of receptors or endogenous ligand would lead to changes in the response to exogenous HRG. In this study, we investigate the activity of rHRG β1, administered in vivo by ALZA pump (ALZA Corp., Mountain View, CA) through-
out 14 days, in these gene-targeted mouse lines, as well as in pregnant and nonpregnant female WT mice. We demonstrate that in WT and HRG(+/-) mice, the effects of rHRG β1 treatment are severe, as evidenced by a high mortality rate. The decrease in receptor levels in ErbB2(+/-) and ErbB3(+/-) mice and the pregnant state of WT females seems to diminish the effects of rHRG β1 treatment, resulting in a significantly lower mortality rate. rHRG β1-induced mortality seems to involve the gastrointestinal system, where a loss of parietal and chief cells is seen, as well as possible motility defects. Histological analysis reveals hyperplasia in epithelial target tissues, along with the growth of cardiac peripheral nerve bundles in all genotypes. Interestingly, breast hyperplasia was extensive in all genotypes but was confined to the female. This finding, along with the protective effect of pregnancy, suggests that the female hormonal environment can influence the activity of HRG and the ErbB family.

Materials and Methods

Experimental Animals

Chimeric mice were generated by conventional gene targeting. Mice were bred on C57BL/6J and BALB/c mouse strains with no differences noted in rHRG β1 response because of background strain or backcross level. All animal care was in accordance with NIH guidelines.

Experimental Design/Test Compound

Adult 8- to 12-week-old mice of each genotype, with an average weight of 20 g, were treated with a sustained 14-day systemic delivery of rHRG β1 (amino acids 177 to 244), a soluble form of the EGF-like domain common to all HRG isoforms, using ALZA pumps. The EGF-like domain is a critical part of the HRG molecule, as it alone can stimulate tyrosine phosphorylation of ErbB2, resulting in such downstream signaling effects as acetylcholine receptor synthesis in cultured myotubes. The rHRG β1 form of the EGF-like domain, used in this study, seems to exhibit higher binding affinity in ErbB2-expressing cells than the α form and has increased potency in activating PI-3-kinase in nontransformed human mammary epithelial cells.

Each group that received rHRG β1 consisted of six female and six male mice. Control groups for each genotype (two females and three males) received phosphate-buffered saline (PBS) (Gibco catalog no. 14190-144; Gibco, Gaithersburg, MD)). ALZA miniosmotic pumps, model 2002 (pumping rate, 0.5 µL/hr; duration, 14 days; reservoir volume, 200 µL), were filled, as per ALZA instructions, with rHRG β1 (amino acids 177 to 244), formulated, and diluted in PBS for a delivered dose of 0.75 mg/kg/day or 1.0 mg/kg/day. Pumps were stored at 4°C overnight in PBS, before sterile implantation. Animals were anesthetized with ketamine 75 to 80 mg/kg, xylazine, 7.5 to 15 mg/kg and acepromazine (all from Fort Dodge Laboratories, Fort Dodge, IA), 0.75 mg/kg, delivered intraperitoneally. The filled pump, delivery portal first, was inserted into a subcutaneous pocket along the back. Animals were individually housed and observed daily. Any moribund animals were immediately sacrificed and necropsied.

Histopathology

Surviving animals were sacrificed and necropsied at day 14. Serum was collected, centrifuged, and frozen for clinical chemistry determinations and kinase receptor assay (KIRA) rHRG β1 analysis; bone marrow smears were also prepared. Organs were fixed in 10% neutral-buffered formalin (Richard Allen Scientific, Kalamazoo, MI) at 22°C overnight, followed by storage in 70% ethanol. For paraffin embedding, tissues were dehydrated through graded alcohols, followed by methyl salicylate, and overnight infiltration in Paraplast at 57°C. Serial 6-µm sections were cut and affixed to polylysine-coated slides before hematoxylin and eosin staining and histological analysis.

Statistical Methods

Mortality was compared among the four genotypes and between the pregnant and nonpregnant WT females using the Pearson chi-square test and Kaplan-Meier cumulative survival plots. After log-transformation (log10 pmol/L) of the raw serum rHRG β1 concentrations to reduce variance heterogeneity, concentrations were compared by genotype and sex, or by pregnancy and nonpregnancy, using analysis of variance. If an interaction between genotype and sex was observed, the relationship between rHRG β1 concentration and genotype was assessed separately for males and females. Pairwise comparisons between genotypic groups was done using the Fisher protected least significance difference procedure for multiple comparisons.

Results

Using gene targeting, heterozygous (+/-) mice were created that lacked one of the functional copies of the HRG-1, ErbB2, or the ErbB3 gene. These mice were viable and fertile, yet had altered levels of their respective targeted gene product, providing a model system to study the interactions of HRG, ErbB2, and ErbB3. In the current study, we challenged these mice with 0.75 mg/kg/day rHRG β1 throughout a 14-day period to investigate effects of receptor and endogenous HRG levels on in vivo rHRG β1 activity.

Overall Effects of rHRG β1 Treatment Based on Genotype (Morbidity and Mortality)

Tolerance for rHRG β1 treatment varied depending on genotype. Mortality differed significantly between the genotypes (P < 0.001) with the HRG(+/-) group having the highest mortality (12 of 12; 100%), the ErbB2 and ErbB3(+/-) groups having the lowest mortality (1 of 12;
were significantly lower than those in the ErbB3(+/−), HRG(+/−), and WT groups (Figure 1B). No relationship was noted between rHRG β1 concentration and genotype in males.

Clinical chemistries were also performed on serum collected at necropsy. Although there were trends toward higher values in the clinical chemistry profiles of rHRG β1-treated animals (cell/liver injury (aspartate aminotransferase, alanine aminotransferase), pancreatic injury (amylase), liver injury (alkaline phosphatase), muscle injury (creatine kinase), hepatobiliary system (γ-glutamyl transpeptidase), kidney injury (blood urea nitrogen and creatinine), hepatobiliary system (total bilirubin), electrolytes (Ca, Na, K, Cl), and phosphorus), most were within established normal ranges. Sporadic elevations of alanine aminotransferase, aspartate aminotransferase, creatine kinase, and amylase were noted in some rHRG β1-treated WT and HRG(+/−) animals, as well as lowered glucose levels relative to controls, probably reflective of their poor condition.

Hematology and urine evaluations were not performed because of the small sample sizes available for analysis from the ill mice. The brain, heart, kidney, and liver were weighed in selected mice with no significant differences found because of rHRG β1 treatment. Bone marrow smears showed evidence of trilinear cellularity indicating no obvious abnormalities and no differences were noted between the groups in this study.

Breast Tissue

Differential expression of the four ErbB receptor family members and distinctive patterns of expression of ErbB ligands have been reported for the different stages of mammary gland development, with ErbB2: ErbB3 being the predominant HRG signaling heterodimer.29 Although ErbB2 overexpression has been implicated in 30% of human breast cancers,1,2 the role of HRG in cancer is poorly understood. Interestingly, in 19 of 20 female mice examined that were treated with rHRG β1, extensive breast ductal hyperplasia was present regardless of genotype (Figure 2). Because of early mortality, HRG(+/−) females were only exposed to rHRG β1 for 5 to 6 days in this study, yet they consistently showed breast hyperplasia, illustrating the rapidity of the response (Figure 2c). Surprisingly, breast ductal hyperplasia was observed only in female mice, with male mice primarily unaffected. In fact, except for two males, one ErbB2(+/−) and one ErbB3(+/−), which showed a very unusual focal pattern of acinar hyperplasia and squamous metaplasia of the ducts, quite different from the females, there was only slight sporadic ductal proliferation noted in any of the other male mice. Therefore, although there was no effect in breast tissue based on genotype, rHRG β1 activity was profoundly influenced by the sex of the animal. These observations suggest an association between rHRG β1 activity and sex or possibly hormonal status.

Figure 1. Cumulative survival plot analysis, rHRG β1 serum levels from females of the four genotypic groups. A: Male and female wild-type (WT), HRG(+/−), ErbB2(+/−), and ErbB3(+/−) animals were treated with 0.75 mg/kg/day rHRG β1. Mortality varied significantly between the genotypic groups using the Pearson chi-square test (P < 0.001). No difference in mortality was noted based on sex. In the WT group receiving rHRG β1, 7 of the 12 animals (58%) died by days 5 to 6, the remaining five animals all survived the 14-day length of the study. In the HRG(+/−) group receiving rHRG β1, four animals were found dead at days 5 to 6, the remaining eight HRG(+/−) animals in this group were sacrificed on days 5 to 6 because of severe clinical signs. In contrast, 92% of the ErbB2(+/−) and ErbB3(+/−) animals survived the study. B: No relationship was noted between the concentration of rHRG β1 and genotype in males; using analysis of variance, in females rHRG β1 levels in ErbB2(+/−) animals were significantly lower than in the other genotypic groups (P = 0.001). rHRG β1 concentrations were log-transformed (log10 pmol/L) to reduce variance heterogeneity. The scatter plot of log-serum rHRG β1 concentrations in female mice treated with 0.75 mg/kg/day rHRG β1 illustrates comparisons by genotype. The mean for each group is shown as a horizontal line within each plot. All control mice had less than detectable rHRG β1 levels.
Gastric Tissue

Overexpression of ErbB2 is also known to play a role in gastric cancer\(^4\) and there is evidence that HRG\(\alpha\) may play a role in the proliferation of gastric epithelial cells through mesenchymal-epithelial interaction in the gastric mucosa.\(^3\) Treatment of WT and HRG(+/−) mice of both sexes with rHRG \(\beta1\) produced animals with grossly distended abdomens; this abdominal phenotype was associated with early mortality. In contrast, the abdomens in the ErbB2(+/−) and ErbB3(+/−) mice and the five surviving WT mice appeared normal on external examination. At necropsy of nonsurviving rHRG \(\beta1\)-treated WT and HRG(+/−) mice, visible abdominal distention correlated with stomachs filled with undigested food and little fecal formation, suggesting reduced digestion/motility, rather than starvation because of a lack of feeding. On histological examination, these severely affected WT and HRG(+/−) animals exhibited a marked dilation of the stomach with extensive muscle thinning (Figure 3, b and c). No mucosal ulceration was present, however. The

\(\text{Figure 2. Female breast. Sections through the breast tissue from female mice; a representative control WT animal (a) and a WT (b), HRG(+/−) (c), ErbB2(+/−) (d), and ErbB3(+/−) animal (e) that received rHRG \(\beta1\). Sections are from mice that received rHRG \(\beta1\) treatment for 14 days except in the case of the HRG(+/−) that was only dosed for 5 to 6 days. rHRG \(\beta1\) exposure induced a generalized ductal hyperplasia with papillomatosis (arrows) in all groups that received rHRG \(\beta1\), regardless of genotype. Males did not show hyperplasia. H&E; original magnification, ×20.}\)
chief cells, gastric zymogen-producing cells, and parietal cells, gastric acid producers in the stomach were examined in detail. A striking depletion of both lineages was noted in these rHRG β1-treated WT and HRG(+/−) mice, with an increase in the number of surface and undifferentiated epithelial cells moving down into the gastric crypts (Figure 3, b and c).31

In contrast, ErbB2 and ErbB3(+/−) mice exhibited far less severe abdominal abnormalities in response to rHRG β1 (Figure 3, d and e). There was histological evidence of only a mild gastric mucosal hyperplasia and parietal cell and chief cell loss was not as severe as that observed in the nonsurviving WT or HRG(+/−) animals. Additionally, the digestive system in the ErbB2(+/−), ErbB3(+/−), and surviving WT mice seemed to be functional. Fecal material was present in the intestine, suggesting that factors other than just parietal and chief cell loss, such as gut motility, were also implicated in the severely affected WT and HRG(+/−) animals. rHRG β1 treatment also induced a generalized mucosal hypertrophy and hyperplasia in the small and large intestines in all genotypes that was more extensive with prolonged exposure.
During development, HRG, acting through the ErbB2:ErbB4 heterodimer, is required for the morphogenesis of cardiac myocyte trabeculae as shown by gene-targeting experiments. In contrast, ErbB3 expression is limited to the cardiac cushions and ErbB3 null mice die in mid-gestation likely because of abnormal cardiac cushion formation. Interestingly, recent studies have shown that whereas ErbB3 expression is dramatically down-regulated, HRG, ErbB2, and ErbB4 continue to be expressed in adult rat myocardium and play a role in cardiac myocyte proliferation, hypertrophic growth and survival in vitro. In this study, heart tissue was sporadically affected by rHRG β1 treatment in all four genotypes with focal subendocardial areas of necrosis and with macrophage accumulation. These lesions were not present in any control animals. Interestingly, mice treated with rHRG β1 also showed changes in the heart valves, characterized by an increase in the amount of extracellular matrix proteoglycans in the valve leaflets in sporadic animals of all four genotypes as compared to controls (data not shown). However, the most striking effect of rHRG β1 treatment in the heart was the presence of numerous peripheral nerve bundles just under the epicardial mesothelium in all four genotypes (Figure 4). Because none was noted in the control groups, the growth and enlargement of these nerve bundles seem to be a direct response to the action of rHRG β1. Further analysis is ongoing to determine the origin of these nerve bundles and associated ErbB expression, because peripheral nerves are known to produce HRG and Schwann cells of developing peripheral nerves are a target for HRG.

Pancreas

The pancreas appeared primarily normal in the treated nonsurviving WT and HRG(+/−) mice at necropsy, with limited ductal ectasia and minimal hyperplasia, probably reflecting the short exposure of the animals to rHRG β1 (5 to 6 days) (Figure 5, b and c). In contrast, in the ErbB2 and ErbB3(+/−) animals exposed for the full 14 days, there was pronounced ductal hyperplasia and proliferation in the main pancreatic ducts at necropsy with inflammatory cells present in the lumen of the ducts (Figure 5, d and e). Acinar cell injury was not widespread, however, although amylase levels were elevated in many of the animals.

Other Tissues

Epithelial proliferation was noted in the genitourinary system (primarily the urinary bladder), the liver bile ducts, and the salivary glands in all groups receiving rHRG β1 treatment. Some animals also had a slight degree of hyperplasia of the epithelium lining the reproductive tract. WT, HRG(+/−), and ErbB3(+/−) mice, treated with rHRG β1, exhibited extensive thymic atrophy and slight splenic atrophy with lymphoid depletion; rHRG β1-treated ErbB2(+/−) mice were the least affected with most thymus tissue appearing normal. No effect of rHRG β1 treatment was seen in the brain, adrenal glands, skin, lungs, or kidneys in this study and no necrosis or inflammation was noted in sciatic nerve of any animals. No significant organ weight changes in the brain, heart, kidney, or liver were noted in the rHRG β1 versus control animals.
Overall Effects of rHRG β1 Treatment Associated with Pregnancy (Morbidity and Mortality)

Based on the sensitivity of the female breast to rHRG β1, we decided to examine whether hormonal influences could play a role in the differential sensitivity of the breast tissue to rHRG β1. Two groups of WT female mice, nonpregnant and pregnant, were treated with 1 mg/kg/day rHRG β1 by ALZA pump for 14 days, beginning on day 7.5 of pregnancy. A slightly higher dose of rHRG β1 was used in this experiment to better discern differences between these two WT groups. Surprisingly, mortality was significantly higher in the nonpregnant mice (14 of 14) than the pregnant mice (2 of 8) (P < 0.001) (Figure 6A). Some pregnant mice delivered viable pups that, on histological examination, showed no anomalies, indicating pregnancy somehow lessened the severity of effects with rHRG β1 treatment. Serum was again collected at necropsy and analyzed by KIRA to determine rHRG β1 levels. Although all control mice had undetectable levels of rHRG β1, serum rHRG β1 was significantly lower in pregnant mice than in nonpregnant mice with a P value of 0.011 (Figure 6B). Of the two pregnant mice that died...
ErbB2, ErbB3, or HRG heterozygous mouse lines provide a unique experimental system to assess the activity of exogenous rHRG β1 treatment. In this study, female mice were found to be uniquely responsive to rHRG β1 treatment, with a significant relationship between circulating levels of rHRG β1 and genotype; rHRG β1 levels in the female ErbB2(+/−) group were significantly lower than in the other genotypes. In addition, the mammary gland exhibited an extensive hyperproliferation in response to rHRG β1 regardless of genotype, but again only in female mice. This dramatic effect in the mammary gland correlates with previous reports where HRG was targeted to the breast via the murine mammary tumor virus promoter promoter, resulting in terminal end-bud structures persisting in the gland of the virgin female transgenics; when bred, these mice later developed mammary adenocarcinomas. Additionally, when HRG was introduced in the breast of mice via Elvax pellets, induction of epithelial ductal branching occurred with the overall response increased by exogenous estradiol and progesterone.

We also determined that rHRG β1 effects in WT female mice were likely influenced by pregnancy, as circulating levels of rHRG β1 were lower than in nonpregnant females, correlating with their reduced mortality. During pregnancy, this reduction in circulating rHRG β1 may be because of expansion of its distribution volume, an increased clearance rate of rHRG β1, or the hormonal status of the animal per se. Both estrogen and the estrogen receptor have been implicated in the regulation of ErbB2 signaling in numerous studies; ErbB2 overexpression is associated clinically with estrogen receptor-negative status and thus resistance to hormonal treatment with antiestrogens such as tamoxifen. Studies of the ErbB2 promoter have identified a 409-bp site in intron-1 that confers estrogen suppression on the ErbB2 promoter in transfection assays and it has also been recently reported that transcription of ErbB2 is negatively regulated by estrogen in breast cancer cells in vitro. Accordingly, the elevated estrogen levels in the pregnant females could potentially contribute to the lessened effects noted with rHRG β1 treatment. Response of ovariectomized females would add significantly to our under-
standing of the mechanism, as would studies of the pharmacokinetics of rHRG β1 clearance.

It has recently been reported that transcripts of all four ErbB receptor family members are expressed in the murine breast during early, mid, and late pregnancy, with HRG-1 transcripts paralleling those of ErbB3 and ErbB4.29 Although endogenous phosphorylation of all four receptors was detectable at late pregnancy and during lactation, ErbB3 and ErbB4 were specifically unresponsive to ectopic EGF or HRG during late pregnancy, suggesting a correlation with the lessened effects noted with rHRG β1 treatment during pregnancy in our study.29

A profound effect of rHRG β1 treatment on gastric tissue was noted in this study, particularly in the nonsurviving WT and HRG(+/-) mice, that exhibited enlarged abdomens filled with undigested food. And, although hyperplasia of the stomach/intestinal epithelium and destruction of the parietal cells was observed in all four genotypes after rHRG β1 treatment, the effects were considerably less severe in surviving WT mice and ErbB2(+/-) and ErbB3(+/-) animals. In accordance with our observations, ErbB2 expression is reported in the epithelium of the intestine and colon30 and low level expression of ErbB2 is also present in both the stomach epithelium30 and in the parietal cells in adulthood. Similarly, ErbB3 is localized in the glandular epithelial cells throughout the gastrointestinal tract30 as well as in the parietal cells.37 EGFR and its associated ligands, including EGF and transforming growth factor (TGF)-α, are also expressed throughout the gastrointestinal epithelium.30 In addition, HRG expression has been reported in the mesenchyme of the embryonic stomach33,48 and HRGα was found by reverse transcriptase-polymerase chain reaction in human gastric fibroblasts and by immunohistochemistry in fibroblasts in the lamina propria in human gastric tissue.30 When HRGα signaling was examined in MKN-28 gastric cancer cells, HRGs was found to induce heterodimerization of ErbB2 and ErbB3 with EGFR, as well as ErbB2 with ErbB3.30 Therefore, a decrease in either ErbB2 or ErbB3 could lead to less efficient HRG β1 signaling in the parietal cells, resulting in the less severe parietal cell pathology in the ErbB2 and ErbB3(+/-) mice compared to WT and HRG(+/-) mice.

Similarly, overexpression of TGF-α caused aberrant gastric mucosal growth as well as parietal cell and chief cell depletion without loss of precursor cells or significant cell death.49 When the effects of rHRGα, TGF-α, and EGF on tyrosine phosphorylation were compared in MKN-28 gastric cancer cells, TGF-α and EGF both stimulated tyrosine phosphorylation of EGFR and ErbB2, whereas HRGα stimulated EGFR, ErbB2, and ErbB3 with the expected association of PI-3 kinase with ErbB3.30 These data suggest that, in the parietal cell phenotype, ErbB2 signaling can be associated with both TGF-α and HRG β1 overexpression.

Gene targeting experiments first revealed that HRG, signaling through ErbB2 and ErbB4, was required for the formation of ventricular trabeculae during cardiac development.21–25 The continued adult expression of HRG, ErbB2, and ErbB4 in adult rat myocardium may play a role in the response of the myocardium to stress.32 Numerous large peripheral nerve bundles that appeared under the epicardial mesothelium were found in most mice treated with rHRG β1 in all four genotypes. Although HRG is a known mitogen and survival factor for Schwann cells,34,35,50 it is intriguing that no similar histological changes were noted in sciatic nerve in these mice.

This investigation describes results of a careful examination of the in vivo activity of rHRG β1 treatment in mice heterozygous for HRG, ErbB2, or ErbB3 allowing the discrimination of differences in the activity of rHRG β1 based on endogenous receptor and HRG levels. rHRG β1 treatment effects were particularly interesting in female animals. Because of the reported cross-talk between the estrogen receptor and the ErbB2 receptor pathways, we also examined the interactions of pregnancy and rHRG β1 activity in WT female mice and found that pregnancy played a protective role in our studies. Also, animals treated with rHRG β1, regardless of genotype, exhibited hyperplasia in many epithelial target tissues where ErbB2 overexpression has been implicated in adulthood cancers such as breast, pancreatic, and gastric cancer. Our findings indicate that HRG might also prove to play a role in the development of such cancers and suggest a possible future therapeutic potential for HRG antagonists.

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