Published in final edited form as:

Hear Res. 2009 June ; 252(1-2): 61–70. doi:10.1016/j.heares.2009.01.012.

Cellular targets of estrogen signaling in regeneration of inner ear sensory epithelia

Jennifer S. McCullar and Elizabeth C. Oesterle*

Virginia Merrill Bloedel Hearing Research Center, Department of Otolaryngology-Head and Neck Surgery, University of Washington, CHDD CD176, P.O. Box 357923, Seattle, WA 98195, USA

Abstract

Estrogen signaling in auditory and vestibular sensory epithelia is a newly emerging focus propelled by the role of estrogen signaling in many other proliferative systems. Understanding the pathways with which estrogen interacts can provide a means to identify how estrogen may modulate proliferative signaling in inner ear sensory epithelia. Reviewed herein are two signaling families, EGF and TGF β . Both pathways are involved in regulating proliferation of supporting cells in mature vestibular sensory epithelia and have well characterized interactions with estrogen signaling in other systems. It is becoming increasingly clear that elucidating the complexity of signaling in regeneration will be necessary for development of therapeutics that can initiate regeneration and prevent progression to a pathogenic state.

Keywords

Estrogen; EGF; TGF α ; TGF β ; Regeneration; Cochlea; Review

1. Introduction

Hearing loss affects over 31 million Americans and this number is expected to grow substantially as the population ages (Pleis and Lethbridge-Cejku, 2006). Hearing deficits can lead to reduced physical and psychological well-being, depression, and isolation resulting in serious social and economic consequences (Liu and Yan, 2007; Moeller, 2007; Moeller et al., 2007). While hearing loss has a multifactor etiology, death of sensory hair cells is a common denominator in many forms of hearing impairment. Elucidation of signaling pathways involved in hair cell regeneration will help to provide tools for stimulating new hair cell production in the mature cochlea. Described herein is the current understanding of one such pathway, the estrogen signaling pathway, within the context of regeneration of mature auditory and vestibular sensory epithelia.

Originally characterized for its role in reproduction and gender differences, estrogen is emerging as a key player in stem cell differentiation, neuronal protection and regeneration research (Cao and Zhang, 2007; Gottfried-Blackmore et al., 2007). Discovery of these new roles has heightened efforts to delineate estrogen's signaling mechanisms and determine how estrogen interacts with other signaling pathways. Estrogen initiates signaling through both membrane and nuclear receptors (Nilsson et al., 2001; Barkhem et al., 2004; Cheskis et al., 2007) and has a diverse repertoire of intracellular regulatory roles (Coleman and Smith, 2001; Kelly and Levin, 2001). Localized synthesis of estrogen creates niches of spatially

*Corresponding author. Tel.: +1 206 616 2705; fax: +1 206 221 5685. oesterle@u.washington.edu (E.C. Oesterle).

restricted activity that contribute to its contextual signaling specificity (Chen et al., 2005; Lambard et al., 2005; Cornil et al., 2006; Leary and Dowsett, 2006; Ohnemus et al., 2006) and varied mechanisms by which estrogen can initiate signaling add to the complexity of the roles for estrogen in biological systems.

2. Mechanisms of estrogen signaling

Estrogens are hormonally active molecules synthesized from androgens *in situ* by aromatase-mediated activity. The most abundant form of estrogen is 17- β -estradiol, which can be converted to estrone and, minimally, to estriol. The estrone and estriol metabolites have stronger affinities for estrogen receptors, but are weaker agonists than 17- β -estradiol (Kuiper et al., 1997). Estrone and estriol have limited, tissue-specific roles in signaling (Ding and Zhu, 2008; Watson et al., 2008), whereas 17- β -estradiol (hereafter “estrogen”) is the best-understood ligand. Estrogen is a lipid-soluble hormone that diffuses readily through the plasma membrane to bind to the nuclear receptors, estrogen receptor- α (ER α) and estrogen receptor- β (ER β). ER α and ER β are characterized by highly conserved DNA-binding (97%) and ligand-binding domains (60%). In contrast, the amino-terminal domain of the receptors share only 18% sequence homology and vary in length. This latter region is thought to be partially responsible for the signaling specificity of the ER isoforms (Hall et al., 2001; Heldring et al., 2007). In humans, there are three identified ER α isoforms (ER α 1-3) and five ER β isoforms (ER β 1-5). ER α isoforms 2 and 3 have not been identified outside cancer cell lines, leaving some speculation as to the existence of these splice variants *in vivo*. Splice variants of ER β are expressed differentially in tissues and can modulate ER α signaling (Ogawa et al., 1998; Matthews and Gustafsson, 2003; Ramsey et al., 2004; Leung et al., 2006). For example, the ER β 2 isoform can inhibit ER α -dependent transcription when both receptors are expressed in the same cell (Matthews and Gustafsson, 2003). Beyond ER β 2, little else is known about the functions of other ER β isoforms. For the purpose of this review, ‘ER α ’ and ‘ER β ’ will refer to generalized receptor functions rather than tissue-specific isoforms.

Nuclear ER α and ER β can be activated in a ligand-dependent and ligand-independent manner (Fig. 1). In ligand-dependent activation, heterodimerized ligand-bound receptors elicit direct genomic effects by binding an estrogen response element (ERE) in the promoter region of target genes. A conservative estimate of 600 possible target genes was reported from a screen for genes responsive to estrogen in the human genome, illustrating a wide array of genes potentially regulated by estrogen. Of these genes, many had putative EREs in their promoter regions for direct regulation of transcription by estrogen (Tang et al., 2004). However, nearly 35% of genes responsive to changes in estrogen are modulated through indirect regulation of transcription, where the receptor dimers bind and regulate other transcription factors (Marino et al., 2006). Direct and indirect activation of genes responsive to estrogen relies on recruitment of co-factors specific to the cellular signaling context. Non-genomic effects of estrogen signaling that modulate cytoplasmic or cell membrane-bound regulatory proteins, independent of gene transcription or protein synthesis, can also be activated in a ligand-dependent manner.

In ligand-independent ER activation, activation of kinases by other signaling pathways results in phosphorylation and activation of ERs. The consequences of ER activation by phosphorylation are similar to that of ligand-mediated activation, with both genomic, and non-genomic functions (reviewed in Weigel and Zhang, 1998; Auger, 2001; Coleman and Smith, 2001; Osborne et al., 2001; Flint et al., 2002; Hart and Davie, 2002; Cvaro et al., 2006). Ligand-independent activation of ERs has been described for growth factors (Pietras et al., 1995; El-Tanani and Green, 1997; Marquez et al., 2001; Ma et al., 2007; Berno et al., 2008; Sinkevicius et al., 2008), dopaminergic agonists (Power et al., 1991; Blaustein, 2004; Cheskis et al., 2007), peptide hormones (e.g., GnRH) (Demay et al., 2001; Pak et al., 2006; Morales et al.,

2007), cyclin-dependent kinase (Lecanda et al., 2007), protein kinase A, protein kinase C (Chen et al., 1999; Longo et al., 2006; Al-Dhaheri and Rowan, 2007) and other activators of cell signaling pathways (e.g., MAPK) (Kato et al., 1995; Bunone et al., 1996).

Although best characterized as nuclear receptors, limited expression of both ER α and ER β occurs at the cell membrane. Signaling initiated by these membrane receptors is thought to contribute to non-genomic activity of estrogen by altering growth factor signaling and contributes to genomic functions by translocating as a ligand–receptor complex to the nucleus for ERE binding (Pappas et al., 1995; Levin, 1999; Clarke et al., 2000). Another membrane estrogen receptor is the recently discovered G-protein-coupled receptor, GPR30. Identification of this receptor helped solve the long-standing mystery of how the rapid non-genomic second messenger signaling of estrogen was mediated (Carmeci et al., 1997). GPR30 binds β -estradiol with the same affinity as ER α (Revankar et al., 2005). Transfection of GPR30 into cell lines and activation with estrogen, alters adenylyl cyclase activity (Thomas et al., 2005) and has been linked to transactivation of epidermal growth factor (EGF) receptors (Filardo, 2002). However, many uncertainties remain regarding the functions of this newly discovered estrogen receptor. Such as the potential interplay between GPR30 signaling and the traditional nuclear ERs, and details of the signaling mechanisms both at the membrane (Filardo and Thomas, 2005; Thomas and Dong, 2006; Watson et al., 2006; Filardo et al., 2007) and intracellularly (Matsuda et al., 2008; Teng et al., 2008; Wang et al., 2008).

3. Estrogen signaling in proliferation and regeneration

Stem cells maintain the ability to divide and differentiate into specialized cells. Both human and mouse embryonic stem cells are estrogen-responsive, as are multiple types of stem-like progenitor cells (e.g., hematopoietic stem cells, neural stem cells), prompting a focus on estrogen signaling in stem cell differentiation and regeneration research (Korach, 1994; Son et al., 2005; Hong et al., 2006; Gilliver et al., 2007; Hong et al., 2007). Estrogen treatment in a mouse embryonic stem cell line promoted cell proliferation and an up-regulation of both ER α and ER β , suggesting a direct effect of estrogen signaling on embryonic stem cell cycle regulation (Han et al., 2006). Estrogen increased proliferation of both glial cells and neural stem cells in neural cultures from embryonic rat (Brannvall et al., 2002). Similarly, estrogen increased proliferation of neuronal cultures taken from both male or female chicks (Cao and Zhang, 2007), suggesting that localized proliferative effects of estrogen are gender-independent. In addition, there are significant data regarding estrogen effects on proliferation in mature systems. For example, estrogen treatment after carotid artery injury in mature rats was found to promote both proliferation and migration of endothelial cells. These effects are partially attributable to an estrogen-dependent increase in vascular epidermal growth factor (VEGF), which promotes endothelial cell proliferation and re-endothelialization *in vivo* (Shifren et al., 1996; Concina et al., 2000; Kim and Levin, 2006). Although not correlated with estrogen activity, increased cochlear VEGF expression has been observed in the stria vascularis and in spiral ganglion cells of noise-exposed animals (Picciotti et al., 2006). Contributions of estrogen signaling to repair and recovery of mature epithelia by regulating proliferation has also been identified in pancreatic β -cells, cartilage, uterine epithelium and hippocampal neurons (Moraghan et al., 1996; Tanapat et al., 1999; Nephew et al., 2000; Talwar et al., 2006; Cano et al., 2008). Evidence is accumulating that the mechanisms for estrogen signaling in repair and recovery of other systems may extend to the mature inner ear.

4. Estrogen signaling in the peripheral auditory system

Clinical hearing research for a role of estrogen in hearing has to contend with confounding factors such as presbycusis, differences in occupational noise exposure and combinatorial hormone replacement therapies. While these studies have not identified a direct role for

estrogen in hearing, they do implicate estrogen in hearing function (Jonsson et al., 1998; Hultcrantz et al., 2006; Baraldi Gdos et al., 2007; Dreisbach et al., 2007). Men have consistent estrogen production throughout life, whereas women have steep cyclical changes in estrogen production, depending on the phase of the menstrual cycle, that ranges from concentrations equal to men to those 10-fold higher. After menopause estrogen production in ovaries ceases, and serum concentrations of estrogen for both men and women are essentially the same (reviewed in Cornil et al., 2006). This change provides a unique opportunity to study effects of estrogen on hearing loss by investigating changes in hearing pre- and post-menopause, the effects of estrogen in hormone replacement therapy or by comparing gender differences pre- and post-menopause with age-matched male counterparts. A study comparing mean air-conduction thresholds in post-menopausal women who were receiving either estrogen therapy alone, combined estrogen and progesterone therapy, or no hormone replacement therapy for an average duration of 4 years, showed a 10 dB shift at low frequencies in women receiving estrogen alone and 5 dB shift in women on combinatorial therapy, concluding that estrogen may slow hearing loss in aging women (Kilicdag et al., 2004). Another expansive epidemiological study correlated low serum estrogen levels in post-menopausal women with significant hearing loss when compared to post-menopausal women with no hearing loss. Hearing loss in this study was defined as a 40 dB hearing level at 1000 and 2000 Hz in one ear or both ears. Serum estrogen was 8.4 ± 3.8 pg/ml for patients with hearing loss versus 9.9 ± 8.7 pg/ml in women with normal hearing (Kim et al., 2002), supporting a role for estrogen in hearing protection. These findings were supported by another study showing an improvement in hearing threshold levels at low frequencies by 3–5 dB in post-menopausal women who received any hormone replacement therapy (HRT; estrogen alone or estrogen with progesterone). Further analysis showed post-menopausal women who were not on HRT also had a poorer hearing at low frequencies (3–5 dB loss) when compared to pre- and peri-menopausal women (Hederstierna et al., 2007). However, other studies show no effect of HRT on hearing protection (reviewed in Hultcrantz et al., 2006), which may be due in part to the antagonizing effects of progesterone and estrogen *in vivo* (Guimaraes et al., 2006) or the observation that protection from hearing loss may have only been seen in patients that suffered significant acoustic insult. Estrogen has been implicated in establishing gender differences in hearing during development prior to the onset of menses and ovarian estrogen production (McFadden, 2002; Guimaraes et al., 2004). Until recently much of the ongoing cell signaling research did not include gender as a variable, which may have masked possible estrogen effects relevant to normal auditory physiology.

The above clinical data demonstrate a positive effect of estrogen on hearing when at physiological concentrations. However, there have been reports of negative effects of estrogen on hearing. Several case studies describe reversible and irreversible hearing loss associated with oral contraceptive use (Okulicz, 1978; Hanna, 1986) and sudden temporary hearing loss associated with HRT (Strachan, 1996). These deficits in hearing have been attributed to thrombosis in the short term and/or otosclerotic bone loss in the inner ear over the long term. Because interplay between estrogen and other hormones contributes to the progression of these disorders, it is difficult to decipher the specific role of estrogen signaling in these instances. In guinea pig, long-term elevated non-physiological concentrations of estrogen resulted in severe hearing loss in some animals that was attributable to otosclerosis. Histological examination of those animals also revealed a thinning of the stria vascularis and loss of stereociliary bundles in the middle and apical turns of the cochlea (Horner et al., 2007).

In addition to the clinical data supporting a physiological role for estrogen in hearing, there is molecular evidence that estrogen may have a direct role in maintenance or repair of inner ear sensory epithelia. ER α is present during inner ear organogenesis in mouse (Sajan et al., 2007), and ERs are expressed in normal adult rat and mouse inner ear sensory epithelia and non-sensory epithelia (Stenberg et al., 1999; Meltser et al., 2008). A microarray study by

Hawkins et al. (2007) detected significant up-regulation of estrogen receptors after either aminoglycoside antibiotic or laser damage of mature chicken auditory and vestibular epithelia. This finding is significant because avian auditory epithelia can regenerate after damage, unlike the mammalian auditory sensory epithelia known as the organ of Corti (OC). Therefore, the up-regulation of ERs seen in chicken suggests estrogen signaling has a role in repair of inner ear epithelia. A recent study by Meltser et al. (2008) was the first study to directly implicate estrogen signaling in recovery of the mammalian OC. This study used temporary threshold shifts to show reduced recovery of hearing thresholds after moderate acoustic trauma in knock-out mice lacking ER α , ER β and aromatase, the enzyme responsible for localized estrogen synthesis. This deficiency was partially restored in the aromatase knock-out mice by treatment with a highly selective ER β agonist, bypassing lack of localized E2 production and stimulating ER β directly. Direct activation of ER α did not affect recovery rates in aromatase knock-out mice and these effects were not found to be gender specific (Meltser et al., 2008). Together, these data implicate estrogen as one of the pathways active in recovery of inner ear epithelia after acoustic damage.

5. Implications for auditory physiology

The sensorineural hair cells are surrounded and sustained by non-sensory supporting cells. There are two mechanisms by which hair cells are replaced: (1) mitotic proliferation, where the supporting cells divide and a daughter cell differentiates into a hair cell (Corwin and Cotanche, 1988; Ryals and Rubel, 1988; Girod et al., 1989; Presson and Popper, 1990) and (2) direct transdifferentiation, a non-mitotic process in which supporting cells directly transform into hair cells without cell division (Raphael et al., 1994; Adler and Raphael, 1996; Roberson et al., 2004). Although it is possible to induce replacement of hair cells via both pathways, direct transdifferentiation may deplete the supporting cells necessary for healthy hair cell function and survival (Izumikawa et al., 2005; Cafaro et al., 2007). In mammals, the OC loses its ability to create new hair cells *in utero* (Chen and Segil, 1999) and the vestibular epithelia (sacculle and utricle) cease hair cell production shortly after birth (Ruben, 1967). However, despite apparent quiescence, adult mammalian vestibular tissues maintain limited regenerative capabilities (Warchol et al., 1993; Lambert, 1994; Rubel et al., 1995; Li and Forge, 1997; Zheng and Gao, 1997; Zheng et al., 1997; Kuntz and Oesterle, 1998; Ogata et al., 1999; Li et al., 2003; Oesterle et al., 2003; Martinez-Monedero et al., 2007; Oshima et al., 2007) that can be stimulated by treatment with growth factors (Lambert, 1994; Yamashita and Oesterle, 1995; Corwin et al., 1996; Zheng et al., 1997; Kuntz and Oesterle, 1998; Oesterle et al., 2003). In contrast, agents to stimulate proliferation in the completely quiescent mature OC remain elusive (Sobkowicz et al., 1992; Roberson and Rubel, 1994; Chardin and Romand, 1995; Sobkowicz et al., 1996; Sobkowicz et al., 1997; Zine and de Ribaupierre, 1998). Ideal methods for restoring lost hair cells would replace hair cells while maintaining supporting cell populations. Promising approaches include transplantation of stem cells, induction of stem-like properties in existing cells, activating transdifferentiation, and stimulating proliferation of existing adult stem cells (reviewed in Collado et al., 2008). Recent studies in the neonatal mouse inner ear have identified a stem cell population in all sensory epithelia and demonstrate that vestibular tissues retain cells with stem-like capabilities throughout adulthood, further suggesting proliferative potential in the mature mammalian ear (Li et al., 2003; Oshima et al., 2007).

Non-pathological proliferation of the inner ear sensory epithelia can be categorized into two processes: developmental proliferation and regenerative proliferation. The extent to which these processes overlap is under investigation. Much of the current work to identify regulators of proliferation has focused on factors that affect proliferation during development or inhibit processes associated with maturation. Recapitulating development to replace hair cells lost to damage has had some limited success. One example shows disruption of the cell cycle control

regulator, p27^{kip}, promoted ongoing proliferation of supporting cells and ectopic expression of cells in the neonatal and adult organ of Corti (Chen and Segil, 1999; Lowenheim et al., 1999). However, the temporal and concurrent signaling necessary to coordinate regulation of p27^{kip} and appropriate localization of replacement hair cells is ongoing. Another more direct approach to replace hair cells is to stimulate native auditory sensory epithelia repair/recovery pathways that can promote division of existing progenitor cells. Due to the complex array of signaling likely to be involved, this is apt to be accomplished through coordinated signaling that will simultaneously relieve inhibition while promoting proliferation of mature inner ear epithelia. Although cross-talk by estrogen signaling pathways in inner ear is a new focus, in many other tissues, estrogen is known to intimately interact with multiple signaling pathways (Imamov et al., 2005; Asarian, 2006; Bodo and Rissman, 2006; Cheskis et al., 2007; Heldring et al., 2007). Hence, we will focus here on the interaction of estrogen with growth factor signaling pathways that are known to be important for regulation of proliferation in the inner ear sensory epithelia. This may serve to provide insights into possible targets for molecular cross-talk between these pathways.

6. Targets for regulation by estrogen in auditory sensory epithelia

Homeostasis is the major energy investment in mature biological systems. Identification of factors that can release homeostatic, controls acting in the OC will be important for activating regenerative proliferation. However, such factors will only be therapeutically effective if repair can be stimulated without causing a novel pathology such as unregulated cell growth or oncogenesis. Therapies need to be developed that release inhibition, promote repair and then re-instate maintenance pathways at the proper time to restore tissues to the homeostatic state. This can be accomplished after better understanding the pathways, cross-talk and temporal organization of the active maintenance and repair signaling networks. Two families of growth factors, the epidermal growth factor (EGF) superfamily and the transforming growth factor β (TGF β) superfamily, have shown promise in shifting adult mammalian inner sensory epithelia away from active maintenance, or quiescence, and into a modest proliferative state. Importantly, and as will be discussed later, both of these pathways interact with estrogen signaling in other systems.

7. ErbB signaling

The EGF receptors are a family of tyrosine kinase receptors that recognize over eleven different ligands (Table 1). There are four classes of EGF receptors, ErbB1-4, which can homo- or heterodimerize. Ligand-binding of ErbB receptors results in post-receptor regulation of a variety of signaling cascades (Riese and Stern, 1998; Yarden and Sliwkowski, 2001; Linggi and Carpenter, 2006). The pathway(s) activated by ErbB signaling are determined by the particular ligand-receptor pair and by the other signaling pathways that are active prior and coincident to ErbB activation. ErbB signaling is well characterized for its contribution to oncogenesis and the cellular effects of activation include cell division, cell death, motility, and adhesion (reviewed in Yarden and Sliwkowski, 2001; and Linggi and Carpenter, 2006). Despite very diverse signaling roles in other tissues, four tested ErbB ligands have been shown to potentiate proliferation of inner ear vestibular sensory epithelia.

The ErbB ligands that potentiate proliferation in mammalian vestibular sensory epithelia include transforming growth factor- α (TGF α , with and without insulin), EGF (with insulin), glial growth factor 2 (GGF2) and heregulin (HRG) (Lambert, 1994; Yamashita and Oesterle, 1995; Zheng et al., 1999; Montcouquiol and Corwin, 2001; Malgrange et al., 2002; Hume et al., 2003; Gu et al., 2007). While all of these ligands elicit mitogenic effects in the vestibular sensory epithelia, their effects differ with maturity and in interactions of ligand with insulin signaling. Regarding age-dependent sensitivity of vestibular tissue to growth factors, GGF2

and HRG stimulate proliferation in neonatal, but not mature, vestibular sensory epithelia. GGF2 and HRG are ligands derived from alternate splicing of the neuregulin-1 gene (Carraway and Burden, 1995), and both modestly potentiate proliferation in cultured neonatal utricles. Increased supporting cell proliferation was seen in dissociated utricular sheet cultures taken from rats before postnatal day 10 and treated with GGF2 or HRG (Zheng et al., 1999; Montcouquiol and Corwin, 2001; Gu et al., 2007). Neonatal organotypic utricular cultures were also sensitive to treatment with HRG (Zheng et al., 1999; Hume et al., 2003). Unlike EGF or TGF α , vestibular sensory epithelia have a restricted temporal sensitivity to GGF2 and HRG. Neither GGF2 or HRG significantly affected proliferation in utricular cultures from 4–6 week old mice (Hume et al., 2003) or rats older than postnatal day 10 (Gu et al., 2007). The finding that treatment of sensory epithelia with GGF2 or HRG has effects limited to young tissue supports the idea that before postnatal day 10, the signaling in rodent utricle may be continuing to mature. This loss of sensitivity to growth factors with continued maturity of the organ, at a stage when vestibular organs are thought to be fully developed (4–6 weeks), suggests a delayed onset of inhibitory regulation in the inner ear. These differences in responsiveness of developing versus mature tissues highlight the need to better understand active homeostatic mechanisms in mature tissues.

Regarding interactions of ErbB ligand with insulin signaling, insulin is a hormone involved in energy homeostasis that is known to modulate growth factor activity in many systems including inner ear sensory epithelia. In organotypic utricular cultures from neonatal mice, combined treatment of GGF2 and insulin increased proliferation by nearly 30% over either factor alone (Gu et al., 2007). In undamaged utricular cultures from mature mice, mitogenic effects of TGF α were potentiated by, but not dependent on, insulin, suggesting that cross-talk between TGF α and insulin signaling is potentially involved in the regenerative response (Yamashita and Oesterle, 1995; Kuntz and Oesterle, 1998). Co-regulation with insulin is also seen in organotypic cultures of mature mouse utricles treated with EGF and insulin concomitantly. Treatments showed a dose-dependent increase in supporting cell proliferation, however unlike TGF α , neither EGF or insulin alone significantly affected proliferation (Yamashita and Oesterle, 1995).

The mechanisms by which the erbB ligands and their co-regulation by insulin affect proliferation in inner ear epithelia are still unknown. These signaling pathways are sites for interaction with estrogen signaling in other regenerative systems, and it is likely that cross-talk between estrogen and ErbB signaling occurs in the ear as well. There are multiple examples of mutual regulation between estrogen and other cell signaling pathways. We describe three in detail below: interactions between estrogen and EGF, TGF α , and TGF β .

8. Estrogen and EGF

One of the first characterized roles for the estrogen receptor GPR30 was the stimulation of EGF production (Filardo, 2002; Filardo et al., 2002; Filardo and Thomas, 2005; Filardo et al., 2008). Estrogen binding of GPR30 activates MAPK signaling cascades via adenylyl cyclase causing the release of heparin-bound EGF from the plasma membrane (Filardo et al., 2000). Despite the signaling role of GPR30 being primarily deduced using cancer cell lines, the role for GPR30 in EGF secretion and proliferation is likely to be relevant to non-pathogenic tissues. In some contexts, estrogen signaling activates EGF and, conversely, EGF can regulate estrogen signaling. For example, EGF initiates ligand-independent activation of estrogen signaling in mouse uterine epithelium, a mature epithelium that undergoes continued cyclical proliferation. This effect is not seen in the ER α knock-out mouse (Hewitt et al., 2005), suggesting that EGF activation is ER α -dependent rather than GPR30 receptor-dependent. Recent data from a knock-in mouse expressing a non-ligand-binding ER α , confirmed that ligand-independent activation of ER α by EGF promotes proliferation of estrogen-sensitive uterine epithelia (Sinkevicius et

al., 2008). In inner ear vestibular sensory epithelia, EGF required insulin to potentiate proliferation. While not established in inner ear sensory epithelia, it is possible that EGF may increase estrogen secretion, which, in turn, stimulates insulin signaling (Asarian, 2006; Roepke et al., 2008) making both factors available for potentiating proliferation. Another example of estrogen affecting insulin secretion in a mature tissue is found in pancreatic β -cells. Treatment with estrogen increased insulin synthesis and secretion however, had no effect on proliferation (Alonso-Magdalena et al., 2008). Studies in mice lacking ER α or aromatase (the enzyme required for localized estrogen synthesis) confirmed that estrogen-induced insulin secretion in β -cells is an ER α mediated effect (Barros et al., 2006; Alonso-Magdalena et al., 2008). If we interpret these data to indicate a possible mechanism for the insulin/EGF effects shown in mouse utricle, a model for potentiating supporting cell proliferation is suggested in which the addition of exogenous insulin bypasses ER-stimulated insulin release to promote proliferation of inner ear sensory epithelia. While there are many potential mechanisms for cross-talk between these factors, it is intriguing to consider that temporally coordinated actions of EGF and estrogen could be elements required for regenerative proliferation of the inner ear sensory epithelia.

9. Estrogen and TGF α

Another example of a possible mechanism for co-regulation in the inner ear is found in the mutual regulation of TGF α and estrogen in neuroblastoma cell lines. The presence or absence of ER α signaling pathways can act as a molecular switch, modifying the cellular response to TGF α . In ER α -positive cells, TGF α treatment blocked proliferation and promoted differentiation. When ER α was absent, TGF α treatment resulted in mitotic proliferation (Vyhldal et al., 2000). Modulation of TGF α by ER α occurs via signal transducer and activation of transcription three (STAT3) signaling proteins (Ciana et al., 2003). Because estrogen modulation of TGF α function is strongly implicated in oncogenesis (Junier, 2000), it is difficult to decipher its normal physiological role. However, these studies suggest that investigation into TGF α signaling in auditory systems when ER α is present, may be useful in understanding how cell fate is determined.

TGF α , EGF and other growth factors can activate receptors and transcription factors common to both ER and ErbB signaling (Nagashima et al., 2008). Possible targets for manipulation of supporting cell proliferation in the auditory and vestibular tissues by combined ER and growth factor signaling include the Ras-activated/mitogen-activated protein kinases (MAPKs) pathway, the STAT pathway, the phospholipase C- γ pathway, and the Akt-activated/phosphatidylinositol (PtdIns) 3-kinase pathway.

10. Estrogen and TGF β

The TGF β superfamily of ligands may also be a target for estrogen signaling in the inner ear sensory epithelia. The TGF β family includes over 35 secreted cytokines including TGF β s, activins, bone morphogenic proteins (BMPs), growth differentiation factors (GDFs) and nodal (Table 2). All of the cytokines have tissue-, cell- and context-specific effects on cell growth and differentiation. TGF β receptors are classified as type I or type II based on sequence homology. There are seven identified type I receptors and five type II receptors that comprise over 10 characterized receptor dimer combinations (de Caestecker, 2004). The ligands that signal through these receptor dimers have varying specificities. Some ligands bind to a single receptor pair, while others are promiscuous and can bind multiple receptor pairs with differing affinities. Activation of heterodimerized receptors results in phosphorylation of one or more of seven receptor associated Smad proteins. These include inhibitory Smads (Smad6/7) and receptor Smads, which are grouped into two families (Smad2/3 and Smad1/5/8) based on sequence homology. Once phosphorylated, the receptor-Smads or inhibitory-Smads associate

with cytoplasmic Smad4, which facilitates translocation of the Smad complex to the nucleus where transcription is initiated or, in the case of Smad6/7, inhibited (Xu, 2006).

TGF β ligands regulate progenitor cell proliferation in mature mammalian tissues predominantly by inhibiting proliferation but can have mitogenic effects (Li et al., 1998; Gandrillon et al., 1999; Pirskanen et al., 2000; McCroskery et al., 2003; Kawase et al., 2004). There are examples of differential effects of TGF β ligands on cell proliferation in inner ear sensory epithelia. TGF β ligands inhibited supporting cell proliferation in utricular sheet cultures from neonatal mice, (Zheng et al., 1997). However, activin, another ligand in the TGF β family, potentiates auditory supporting cell proliferation in mature chicken cochlear ducts (McCullar et al., unpublished data). Multiple studies have demonstrated mutual regulation of the TGF β superfamily and estrogen signaling (Kipp et al., 2007a,b). In breast cancer cells, activin represses estrogen-dependent transcription and conversely, estrogen treatment of these cells decreased activin-dependent transcription (Burdette and Woodruff, 2007). Interactions between these signaling pathways are not limited to cancerous tissues. In mature, normal uterine epithelium an environmental estrogen analog (bisphenol A) decreased expression of the type II activin receptor, Acvr2b, and its ligand, BMP7. Concurrent with down-regulation of Acvr2b and BMP7, there was a significant decrease in apoptotic cells, suggesting that estrogen regulation of activin signaling may affect cell survival. This effect was not seen in estrogen-treated testis or ovary, illustrating the tissue-specific context of the interaction (Kusumegi et al., 2004). Moreover, in normal mouse ovary, estrogen treatment specifically reduced transcription of activin. This transcriptional control on Acvr2a/b signaling by estrogen is mediated through the downstream activin signaling protein, Smad3 (Kipp et al., 2007a,b). However, regulation of Smad 3 is not due to direct binding or repression of Smad3 transcription by estrogen (Cherlet and Murphy, 2007), suggesting that estrogen may be interacting with other proteins in the activin signaling pathway as well. Together, these studies suggest that estrogen affects activin signaling both via altering transcription of ligand and by indirectly regulating the activin signaling protein Smad3. Other demonstrations of TGF β cytokines interacting with estrogen signaling were shown in studies of TGF β in endometrial renewal, wound healing and neural proliferation (Ashcroft et al., 1997; Ashcroft et al., 2003; Kanda and Watanabe, 2005; Buck and Knabbe, 2006; Elliot et al., 2006; Malek et al., 2006; Lecanda et al., 2007; Gargett et al., 2008; Kleuser et al., 2008) and in studies of BMP function in bone metabolism and stem cell proliferation (Yamamoto et al., 2002; Paez-Pereda et al., 2003; Zhou et al., 2003; Zhang et al., 2005; Lau et al., 2006).

11. Summary

We are just beginning to delineate factors that can restrict or stimulate supporting cell proliferation and hair cell replacement in mature inner ear sensory epithelia. Identification of ErbB ligands that affect proliferation of supporting cells in mature vestibular sensory epithelia is a promising start, but is limited to the vestibular sensory epithelia and the effects are small in magnitude. There are many questions that remain to be answered including: What factors are inhibiting cell proliferation in mature inner ear tissues? When do pathways necessary for maintaining supporting cell quiescence become active? What is the sequence of signaling necessary to take a supporting cell from quiescence into mitotic proliferation and differentiation and then return it to quiescence? How many cell-signaling pathways are involved? Work has already begun to collect large databases of genes responsive to injury in both mouse and chicken (e.g., Hawkins et al., 2007; Sajan et al., 2007). These data sets offer valuable clues for identifying new proteins involved in regulation of regeneration pathways. As more signaling pathways relevant to regeneration are discovered, the coregulation that controls homeostasis and regeneration can begin to be worked out. Estrogen signaling is of particular interest as a co-regulator due to known effects on proliferation in other tissues, its implication in survival and/or repair of inner ear, and its overlap with ErbB and TGF β signaling pathways.

Acknowledgments

The authors' research is supported by NIH NIDCD Grant DC005361, NIDCD P30 Grant DC04661, NICHD P30 Grant HD002274 and NIDCD DC003944. The authors would like to thank Drs. Monica Skinner, Allison Coffin, Kelly Owens, Yuan Wang, Nicole Schmitt and Martha Port for thoughtful comments and reading of this manuscript.

References

- Adler HJ, Raphael Y. New hair cells arise from supporting cell conversion in the acoustically damaged chick inner ear. *Neurosci Lett* 1996;205 (1):17–20. [PubMed: 8867010]
- Al-Dhaheeri MH, Rowan BG. Protein kinase A exhibits selective modulation of estradiol-dependent transcription in breast cancer cells that is associated with decreased ligand binding, altered estrogen receptor alpha promoter interaction, and changes in receptor phosphorylation. *Mol Endocrinol* 2007;21 (2):439–456. [PubMed: 17068199]
- Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquie M, Gauthier BR, Nef S, Stefani E, Nadal A. Pancreatic insulin content regulation by the estrogen receptor ER alpha. *PLoS ONE* 2008;3 (4):e2069. [PubMed: 18446233]
- Asarian L. Membrane estrogen receptors and energy homeostasis. *J Neurosci* 2006;26 (44):11255–11256. [PubMed: 17083164]
- Ashcroft GS, Dodsworth J, van Boxtel E, Tarnuzzer RW, Horan MA, Schultz GS, Ferguson MW. Estrogen accelerates cutaneous wound healing associated with an increase in TGF-beta1 levels. *Nat Med* 1997;3 (11):1209–1215. [PubMed: 9359694]
- Ashcroft GS, Mills SJ, Flanders KC, Lyakh LA, Anzano MA, Gilliver SC, Roberts AB. Role of Smad3 in the hormonal modulation of in vivo wound healing responses. *Wound Repair Regen* 2003;11 (6):468–473. [PubMed: 14617288]
- Auger AP. Ligand-independent activation of progesterin receptors: relevance for female sexual behaviour. *Reproduction* 2001;122 (6):847–855. [PubMed: 11732980]
- Baraldi Gdos S, de Almeida LC, Borges AC. Hearing loss in aging. *Rev Bras Otorrinolaringol (Engl Ed)* 2007;73 (1):58–64.
- Barkhem T, Nilsson S, Gustafsson JA. Molecular mechanisms, physiological consequences and pharmacological implications of estrogen receptor action. *Am J Pharmacogenomics* 2004;4 (1):19–28. [PubMed: 14987119]
- Barros RPA, Machado UF, Gustafsson J-Å. Estrogen receptors: new players in diabetes mellitus. *Trends Mol Med* 2006;12 (9):425–431. [PubMed: 16890492]
- Berno V, Amazit L, Hinojos C, Zhong J, Mancini MG, Sharp ZD, Mancini MA. Activation of estrogen receptor-alpha by E2 or EGF induces temporally distinct patterns of large-scale chromatin modification and mRNA transcription. *PLoS ONE* 2008;3 (5):e2286. [PubMed: 18509470]
- Blaustein JD. Minireview: neuronal steroid hormone receptors: they are not just for hormones anymore. *Endocrinology* 2004;145 (3):1075–1081. [PubMed: 14670984]
- Bodo C, Rissman EF. New roles for estrogen receptor beta in behavior and neuroendocrinology. *Front Neuroendocrinol* 2006;27 (2):217–232. [PubMed: 16603234]
- Brannvall K, Korhonen L, Lindholm D. Estrogen-receptor-dependent regulation of neural stem cell proliferation and differentiation. *Mol Cell Neurosci* 2002;21 (3):512–520. [PubMed: 12498791]
- Buck M, Knabbe C. TGF-beta signaling in breast cancer. *Ann NY Acad Sci* 2006;1089:119–126. [PubMed: 17261761]
- Bunone G, Briand PA, Miksicek RJ, Picard D. Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO J* 1996;15 (9):2174–2183. [PubMed: 8641283]
- Burdette JE, Woodruff TK. Activin and estrogen crosstalk regulates transcription in human breast cancer cells. *Endocr Relat Cancer* 2007;14 (3):679–689. [PubMed: 17914098]
- Cafaro J, Lee GS, Stone JS. Atoh1 expression defines activated progenitors and differentiating hair cells during avian hair cell regeneration. *Dev Dyn* 2007;236 (1):156–170. [PubMed: 17096404]

- Cano DA, Rulifson IC, Heiser PW, Swigart LB, Pelengaris S, German M, Evan GI, Bluestone JA, Hebrok M. Regulated beta-cell regeneration in the adult mouse pancreas. *Diabetes* 2008;57 (4):958–966. [PubMed: 18083786]
- Cao A, Zhang C. Sex-specific effects of androgen and estrogen on proliferation of the embryonic chicken hypothalamic neurons. *Endocrine* 2007;31 (2):161–166. [PubMed: 17873328]
- Carmeci C, Thompson DA, Ring HZ, Francke U, Weigel RJ. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. *Genomics* 1997;45 (3):607–617. [PubMed: 9367686]
- Carraway KL, Burden SJ. Neuregulins and their receptors. *Curr Opin Neurobiol* 1995;5 (5):606–612. [PubMed: 8580712]
- Chardin S, Romand R. Regeneration and mammalian auditory hair cells. *Science* 1995;267 (5198):707–711. [PubMed: 7839151]
- Chen D, Pace PE, Coombes RC, Ali S. Phosphorylation of human estrogen receptor alpha by protein kinase A regulates dimerization. *Mol Cell Biol* 1999;19 (2):1002–1015. [PubMed: 9891036]
- Chen P, Segil N. P27(Kip1) links cell proliferation to morphogenesis in the developing organ of Corti. *Development* 1999;126 (8):1581–1590. [PubMed: 10079221]
- Chen S, Ye J, Kijima I, Kinoshita Y, Zhou D. Positive and negative transcriptional regulation of aromatase expression in human breast cancer tissue. *J Steroid Biochem Mol Biol* 2005;95 (1–5):17–23. [PubMed: 15955695]
- Cherlet T, Murphy LC. Estrogen receptors inhibit Smad3 transcriptional activity through Ap-1 transcription factors. *Mol Cell Biochem* 2007;306 (1–2):33–42. [PubMed: 17660955]
- Cheskis BJ, Greger JG, Nagpal S, Freedman LP. Signaling by estrogens. *J Cell Physiol* 2007;213 (3):610–617. [PubMed: 17886255]
- Ciana P, Ghisletti S, Mussi P, Eberini I, Vegeto E, Maggi A. Estrogen Receptor {alpha}, a Molecular Switch Converting Transforming Growth Factor-{alpha}-mediated Proliferation into Differentiation in Neuroblastoma Cells. *J Biol Chem* 2003;278 (34):31737–31744. [PubMed: 12709435]
- Clarke CH, Norfleet AM, Clarke MS, Watson CS, Cunningham KA, Thomas ML. Perimembrane localization of the estrogen receptor alpha protein in neuronal processes of cultured hippocampal neurons. *Neuroendocrinology* 2000;71 (1):34–42. [PubMed: 10644897]
- Coleman KM, Smith CL. Intracellular signaling pathways: nongenomic actions of estrogens and ligand-independent activation of estrogen receptors. *Front Biosci* 2001;6:D1379–D1391. [PubMed: 11578956]
- Collado MS, Burns JC, Hu Z, Corwin JT. Recent advances in hair cell regeneration research. *Curr Opin Otolaryngol Head Neck Surg* 2008;16 (5):465–471. [PubMed: 18797290]
- Concina P, Sordello S, Barbacanne MA, Elhage R, Pieraggi MT, Fournial G, Plouet J, Bayard F, Arnal JF. The mitogenic effect of 17beta-estradiol on in vitro endothelial cell proliferation and on in vivo reendothelialization are both dependent on vascular endothelial growth factor. *J Vasc Res* 2000;37 (3):202–208. [PubMed: 10859479]
- Cornil CA, Ball GF, Balthazart J. Functional significance of the rapid regulation of brain estrogen action: where do the estrogens come from? *Brain Res* 2006;1126 (1):2–26. [PubMed: 16978590]
- Corwin J, Cotanche D. Regeneration of sensory hair cells after acoustic trauma. *Science* 1988;240 (4860):1772–1774. [PubMed: 3381100]
- Corwin J, Warchol M, Saffer L, Finley J, Gu R, Lamber P. Growth factors as potential drugs for the sensory epithelia of the ear. *Ciba Found Symp* 1996;196:167–182. (discussion 182–187). [PubMed: 8866134]
- Cvoro A, Tzagarakis-Foster C, Tatomer D, Paruthiyil S, Fox MS, Leitman DC. Distinct roles of unliganded and liganded estrogen receptors in transcriptional repression. *Mol Cell* 2006;21 (4):555–564. [PubMed: 16483936]
- de Caestecker M. The transforming growth factor-beta superfamily of receptors. *Cytokine Growth Factor Rev* 2004;15 (1):1–11. [PubMed: 14746809]
- Demay F, De Monti M, Tiffocche C, Vaillant C, Thieulant ML. Steroid-independent activation of er by gnRH in gonadotrope pituitary cells. *Endocrinology* 2001;142 (8):3340–3347. [PubMed: 11459776]
- Ding J, Zhu BT. Unique effect of the pregnancy hormone estriol on antigen-induced production of specific antibodies in female BALB/c mice. *Steroids* 2008;73 (3):289–298. [PubMed: 18093630]

- Dreisbach LE, Kramer SJ, Cobos S, Cowart K. Racial and gender effects on pure-tone thresholds and distortion-product otoacoustic emissions (DPOAEs) in normal-hearing young adults. *Int J Audiol* 2007;46 (8):419–426. [PubMed: 17654083]
- El-Tanani MK, Green CD. Two separate mechanisms for ligand-independent activation of the estrogen receptor. *Mol Endocrinol* 1997;11 (7):928–937. [PubMed: 9178752]
- Elliot SJ, Karl M, Berho M, Xia X, Pereria-Simon S, Espinosa-Heidmann D, Striker GE. Smoking induces glomerulosclerosis in aging estrogen-deficient mice through cross-talk between TGF-beta1 and IGF-I signaling pathways. *J Am Soc Nephrol* 2006;17 (12):3315–3324. [PubMed: 17093064]
- Filardo E, Quinn J, Pang Y, Graeber C, Shaw S, Dong J, Thomas P. Activation of the novel estrogen receptor G protein-coupled receptor 30 (GPR30) at the plasma membrane. *Endocrinology* 2007;148 (7):3236–3245. [PubMed: 17379646]
- Filardo EJ. Epidermal growth factor receptor (EGFR) transactivation by estrogen via the G-protein-coupled receptor, GPR30: a novel signaling pathway with potential significance for breast cancer. *J Steroid Biochem Mol Biol* 2002;80 (2):231–238. [PubMed: 11897506]
- Filardo EJ, Quinn JA, Bland KI, Frackelton AR Jr. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol Endocrinol* 2000;14 (10):1649–1660. [PubMed: 11043579]
- Filardo EJ, Quinn JA, Frackelton AR Jr, Bland KI. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. *Mol Endocrinol* 2002;16 (1):70–84. [PubMed: 11773440]
- Filardo EJ, Quinn JA, Sabo E. Association of the membrane estrogen receptor, GPR30, with breast tumor metastasis and transactivation of the epidermal growth factor receptor. *Steroids* 2008;73 (9–10):870–873. [PubMed: 18289622]
- Filardo EJ, Thomas P. GPR30: a seven-transmembrane-spanning estrogen receptor that triggers EGF release. *Trends Endocrinol Metab* 2005;16 (8):362–367. [PubMed: 16125968]
- Flint AP, Sheldrick EL, Fisher PA. Ligand-independent activation of steroid receptors. *Domest Anim Endocrinol* 2002;23 (1–2):13–24. [PubMed: 12142222]
- Gandrillon O, Schmidt U, Beug H, Samarut J. TGF-beta cooperates with TGF-alpha to induce the self-renewal of normal erythrocytic progenitors: evidence for an autocrine mechanism. *EMBO J* 1999;18 (10):2764–2781. [PubMed: 10329623]
- Gargett CE, Chan RW, Schwab KE. Hormone and growth factor signaling in endometrial renewal: Role of stem/progenitor cells. *Mol Cell Endocrinol* 2008;288 (1–2):22–29. [PubMed: 18403104]
- Gilliver SC, Ashworth JJ, Ashcroft GS. The hormonal regulation of cutaneous wound healing. *Clin Dermatol* 2007;25 (1):56–62. [PubMed: 17276202]
- Girod D, Duckert L, Rubel E. Possible precursors of regenerated hair cells in the avian cochlea following acoustic trauma. *Hear Res* 1989;42 (2–3):175–194. [PubMed: 2606802]
- Gottfried-Blackmore A, Croft G, Clark J, McEwen BS, Jellinck PH, Bulloch K. Characterization of a cerebellar granule progenitor cell line, EtC.1, and its responsiveness to 17-beta-estradiol. *Brain Res* 2007;1186:29–40. [PubMed: 17980864]
- Gu R, Montcouquiol M, Marchionni M, Corwin JT. Proliferative responses to growth factors decline rapidly during postnatal maturation of mammalian hair cell epithelia. *Eur J Neurosci* 2007;25 (5):1363–1372. [PubMed: 17425563]
- Guimaraes P, Frisina ST, Mapes F, Tadros SF, Frisina DR, Frisina RD. Progestin negatively affects hearing in aged women. *Proc Natl Acad Sci USA* 2006;103 (38):14246–14249. [PubMed: 16959884]
- Guimaraes P, Zhu X, Cannon T, Kim S, Frisina RD. Sex differences in distortion product otoacoustic emissions as a function of age in CBA mice. *Hear Res* 2004;192 (1–2):83–89. [PubMed: 15157966]
- Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *J Biol Chem* 2001;276 (40):36869–36872. [PubMed: 11459850]
- Han HJ, Heo JS, Lee YJ. Estradiol-17beta stimulates proliferation of mouse embryonic stem cells: involvement of MAPKs and CDKs as well as protooncogenes. *Am J Physiol Cell Physiol* 2006;290 (4):C1067–C1075. [PubMed: 16291822]
- Hanna GS. Sudden deafness and the contraceptive pill. *J Laryngol Otol* 1986;100 (6):701–706. [PubMed: 3723005]

- Hart LL, Davie JR. The estrogen receptor: more than the average transcription factor. *Biochem Cell Biol* 2002;80 (3):335–341. [PubMed: 12123286]
- Hawkins RD, Bashiardes S, Powder KE, Sajjan SA, Bhonagiri V, Alvarado DM, Speck J, Warchol ME, Lovett M. Large scale gene expression profiles of regenerating inner ear sensory epithelia. *PLoS ONE* 2007;2:e525. [PubMed: 17565378]
- Hederstierna C, Hultcrantz M, Collins A, Rosenhall U. Hearing in women at menopause. Prevalence of hearing loss, audiometric configuration and relation to hormone replacement therapy. *Acta Otolaryngol* 2007;127 (2):149–155. [PubMed: 17364346]
- Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Strom A, Treuter E, Warner M, Gustafsson JA. Estrogen receptors: how do they signal and what are their targets. *Physiol Rev* 2007;87 (3):905–931. [PubMed: 17615392]
- Hewitt SC, Collins J, Grissom S, Deroo B, Korach KS. Global uterine genomics in vivo: microarray evaluation of the estrogen receptor alpha-growth factor cross-talk mechanism. *Mol Endocrinol* 2005;19 (3):657–668. [PubMed: 15528273]
- Hong L, Colpan A, Peptan IA. Modulations of 17-beta estradiol on osteogenic and adipogenic differentiations of human mesenchymal stem cells. *Tissue Eng* 2006;12 (10):2747–2753. [PubMed: 17518644]
- Hong L, Colpan A, Peptan IA, Daw J, George A, Evans CA. 17-Beta estradiol enhances osteogenic and adipogenic differentiation of human adipose-derived stromal cells. *Tissue Eng* 2007;13 (6):1197–1203. [PubMed: 17518737]
- Horner KC, Cazals Y, Guieu R, Lenoir M, Sauze N. Experimental estrogen-induced hyperprolactinemia results in bone-related hearing loss in the guinea pig. *Am J Physiol Endocrinol Metab* 2007;293 (5):E1224–E1232. [PubMed: 17711987]
- Hultcrantz M, Simonoska R, Stenberg A. Estrogen and hearing: a summary of recent investigations. *Acta Otolaryngol* 2006;126 (1):10–14. [PubMed: 16308248]
- Hume C, Kirkegaard M, Oesterle E. ErbB expression: the mouse inner ear and maturation of the mitogenic response to heregulin. *J Assoc Res Otolaryngol* 2003;4 (3):422–443. [PubMed: 14690060]
- Imamov O, Shim GJ, Warner M, Gustafsson JA. Estrogen receptor beta in health and disease. *Biol Reprod* 2005;73 (5):866–871. [PubMed: 16033996]
- Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, Brough DE, Raphael Y. Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. *Nat Med* 2005;11 (3):271–276. [PubMed: 15711559]
- Jonsson R, Rosenhall U, Gause-Nilsson I, Steen B. Auditory function in 70- and 75-year-olds of four age cohorts. A cross-sectional and time-lag study of presbycusis. *Scand Audiol* 1998;27 (2):81–93. [PubMed: 9638827]
- Junier MP. What role(s) for TGF[alpha] in the central nervous system? *Prog Neurobiol* 2000;62 (5):443–473. [PubMed: 10869779]
- Kanda N, Watanabe S. Regulatory roles of sex hormones in cutaneous biology and immunology. *J Dermatol Sci* 2005;38 (1):1–7. [PubMed: 15795118]
- Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, Chambon P. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science* 1995;270 (5241):1491–1494. [PubMed: 7491495]
- Kawase Y, Yanagi Y, Takato T, Fujimoto M, Okochi H. Characterization of multipotent adult stem cells from the skin: transforming growth factor-beta (TGF-beta) facilitates cell growth. *Exp Cell Res* 2004;295 (1):194–203. [PubMed: 15051502]
- Kelly MJ, Levin ER. Rapid actions of plasma membrane estrogen receptors. *Trends Endocrinol Metab* 2001;12 (4):152–156. [PubMed: 11295570]
- Kilicdag EB, Yavuz H, Bagis T, Tarim E, Erkan AN, Kazanci F. Effects of estrogen therapy on hearing in postmenopausal women. *Am J Obstet Gynecol* 2004;190 (1):77–82. [PubMed: 14749639]
- Kim JK, Levin ER. Estrogen signaling in the cardiovascular system. *Nucl Recept Signal* 2006;4:e013. [PubMed: 16862219]
- Kim SH, Chae HD, Kang BM, Kim CH. The association between serum estradiol level and hearing sensitivity in postmenopausal women. *Obstet Gynecol* 2002;99 (5 Pt 1):726–730. [PubMed: 11978279]

- Kipp JL, Kilen SM, Bristol-Gould S, Woodruff TK, Mayo KE. Neonatal exposure to estrogens suppresses activin expression and signaling in the mouse ovary. *Endocrinology* 2007a;148 (5):1968–1976. [PubMed: 17255206]
- Kipp JL, Kilen SM, Woodruff TK, Mayo KE. Activin regulates estrogen receptor gene expression in the mouse ovary. *J Biol Chem* 2007b;282 (50):36755–36765. [PubMed: 17951260]
- Kleuser B, Malek D, Gust R, Pertz HH, Potteck H. 17- β -Estradiol inhibits Transforming Growth Factor- β signalling and function in breast cancer cells via activation of Extracellular Signal-Regulated Kinase through the G protein coupled receptor 30. *Mol Pharmacol* 2008;74 (6):1533–1543. [PubMed: 18768737]
- Korach KS. Insights from the study of animals lacking functional estrogen receptor. *Science* 1994;266 (5190):1524–1527. [PubMed: 7985022]
- Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 1997;138 (3):863–870. [PubMed: 9048584]
- Kuntz A, Oesterle E. Transforming growth factor alpha with insulin stimulates cell proliferation in vivo in adult rat vestibular sensory epithelium. *J Comp Neurol* 1998;399 (3):413–423. [PubMed: 9733087]
- Kusumegi T, Tanaka J, Kawano M, Yonemoto J, Tohyama C, Sone H. BMP7/ActRIIB regulates estrogen-dependent apoptosis: new biomarkers for environmental estrogens. *J Biochem Mol Toxicol* 2004;18 (1):1–11. [PubMed: 14994273]
- Lambard S, Silandre D, Delalande C, Denis-Galeraud I, Bourguiba S, Carreau S. Aromatase in testis: expression and role in male reproduction. *J Steroid Biochem Mol Biol* 2005;95 (1–5):63–69. [PubMed: 16019206]
- Lambert PR. Inner ear hair cell regeneration in a mammal: identification of a triggering factor. *Laryngoscope* 1994;104 (6 Pt 1):701–718. [PubMed: 8196445]
- Lau KH, Kapur S, Kesavan C, Baylink DJ. Up-regulation of the Wnt, estrogen receptor, insulin-like growth factor-I, and bone morphogenetic protein pathways in C57BL/6J osteoblasts as opposed to C3H/HeJ osteoblasts in part contributes to the differential anabolic response to fluid shear. *J Biol Chem* 2006;281 (14):9576–9588. [PubMed: 16461770]
- Leary A, Dowsett M. Combination therapy with aromatase inhibitors: the next era of breast cancer treatment? *Br J Cancer* 2006;95 (6):661–666. [PubMed: 16926831]
- Lecanda J, Parekh T, Gama P, Lin K, Liarski V, Uretsky S, Mittal K, Gold L. Transforming growth factor-beta, estrogen, and progesterone converge on the regulation of p27Kip1 in the normal and malignant endometrium. *Cancer Res* 2007;67 (3):1007–1018. [PubMed: 17283133]
- Leung YK, Mak P, Hassan S, Ho SM. Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling. *Proc Natl Acad Sci USA* 2006;103 (35):13162–13167. [PubMed: 16938840]
- Levin ER. Cellular functions of the plasma membrane estrogen receptor. *Trends Endocrinol Metab* 1999;10 (9):374–377. [PubMed: 10511697]
- Li H, Liu H, Heller S. Pluripotent stem cells from the adult mouse inner ear. *Nat Med* 2003;9 (10):1293–1299. [PubMed: 12949502]
- Li L, Forge A. Morphological evidence for supporting cell to hair cell conversion in the mammalian utricular macula. *Int J Dev Neurosci* 1997;15 (4–5):433–446. [PubMed: 9263024]
- Li Q, Karam SM, Coerver KA, Matzuk MM, Gordon JI. Stimulation of activin receptor II signaling pathways inhibits differentiation of multiple gastric epithelial lineages. *Mol Endocrinol* 1998;12 (2):181–192. [PubMed: 9482661]
- Linggi B, Carpenter G. ErbB receptors: new insights on mechanisms and biology. *Trends Cell Biol* 2006;16 (12):649–656. [PubMed: 17085050]
- Liu XZ, Yan D. Ageing and hearing loss. *J Pathol* 2007;211 (2):188–197. [PubMed: 17200945]
- Longo M, Peruzzi B, Fortunati D, De Luca V, Denger S, Caselli G, Migliaccio S, Teti A. Modulation of human estrogen receptor alpha F promoter by a protein kinase C/c-Src-dependent mechanism in osteoblast-like cells. *J Mol Endocrinol* 2006;37 (3):489–502. [PubMed: 17170089]
- Lowenheim H, Furness DN, Kil J, Zinn C, Gultig K, Fero ML, Frost D, Gummer AW, Roberts JM, Rubel EW, Hackney CM, Zenner HP. Gene disruption of p27(Kip1) allows cell proliferation in the postnatal and adult organ of corti. *Proc Natl Acad Sci USA* 1999;96 (7):4084–4088. [PubMed: 10097167]

- Ma Y, Hu C, Riegel AT, Fan S, Rosen EM. Growth factor signaling pathways modulate BRCA1 repression of estrogen receptor- α activity. *Mol Endocrinol* 2007;21 (8):1905–1923. [PubMed: 17505062]
- Malek D, Gust R, Kleuser B. 17-Beta-estradiol inhibits transforming-growth-factor-beta-induced MCF-7 cell migration by Smad3-repression. *Eur J Pharmacol* 2006;534 (1–3):39–47. [PubMed: 16497293]
- Malgrange B, Rigo JM, Coucke P, Thiry M, Hans G, Nguyen L, van de Water TR, Moonen G, Lefebvre PP. Identification of factors that maintain mammalian outer hair cells in adult organ of Corti explants. *Hear Res* 2002;170 (1–2):48–58. [PubMed: 12208540]
- Marino M, Galluzzo P, Ascenzi P. Estrogen signaling multiple pathways to impact gene transcription. *Curr Genomics* 2006;7 (8):497–508. [PubMed: 18369406]
- Marquez DC, Lee J, Lin T, Pietras RJ. Epidermal growth factor receptor and tyrosine phosphorylation of estrogen receptor. *Endocrine* 2001;16 (2):73–81. [PubMed: 11887937]
- Martinez-Monedero R, Oshima K, Heller S, Edge AS. The potential role of endogenous stem cells in regeneration of the inner ear. *Hear Res* 2007;227 (1–2):48–52. [PubMed: 17321086]
- Matsuda K, Sakamoto H, Mori H, Hosokawa K, Kawamura A, Itose M, Nishi M, Prossnitz ER, Kawata M. Expression and intracellular distribution of the G protein-coupled receptor 30 in rat hippocampal formation. *Neurosci Lett* 2008;441 (1):94–99. [PubMed: 18586395]
- Matthews J, Gustafsson JA. Estrogen signaling: a subtle balance between ER α and ER β . *Mol Interv* 2003;3 (5):281–292. [PubMed: 14993442]
- McCroskery S, Thomas M, Maxwell L, Sharma M, Kambadur R. Myostatin negatively regulates satellite cell activation and self-renewal. *J Cell Biol* 2003;162 (6):1135–1147. [PubMed: 12963705]
- McFadden D. Masculinization effects in the auditory system. *Arch Sex Behav* 2002;31 (1):99–111. [PubMed: 11910797]
- Meltser I, Tahera Y, Simpson E, Hultcrantz M, Charitidi K, Gustafsson JK, Canlon B. Estrogen receptor β protects against acoustic trauma in mice. *J Clin Invest* 2008;118 (4):1563–1570. [PubMed: 18317592]
- Moeller MP. Current state of knowledge: psychosocial development in children with hearing impairment. *Ear Hear* 2007;28 (6):729–739. [PubMed: 17982361]
- Moeller MP, Tomblin JB, Yoshinaga-Itano C, Connor CM, Jerger S. Current state of knowledge: language and literacy of children with hearing impairment. *Ear Hear* 2007;28 (6):740–753. [PubMed: 17982362]
- Montcouquiol M, Corwin J. Intracellular signals that control cell proliferation in mammalian balance epithelia: key roles for phosphatidylinositol-3 kinase, mammalian target of rapamycin, and S6 kinases in preference to calcium, protein kinase C, and mitogen-activated protein kinase. *J Neurosci* 2001;21 (2):570–580. [PubMed: 11160436]
- Moraghan T, Antoniucci DM, Grenert JP, Sieck GC, Johnson C, Miller VM, Fitzpatrick LA. Differential response in cell proliferation to beta estradiol in coronary arterial vascular smooth muscle cells obtained from mature female versus male animals. *Endocrinology* 1996;137 (11):5174–5177. [PubMed: 8895395]
- Morales A, Gonzalez M, Marin R, Diaz M, Alonso R. Estrogen inhibition of norepinephrine responsiveness is initiated at the plasma membrane of GnRH-producing GT1–7 cells. *J Endocrinol* 2007;194 (1):193–200. [PubMed: 17592033]
- Nagashima T, Suzuki T, Kondo S, Kuroki Y, Takahashi K, Ide K, Yumoto N, Hasegawa A, Toyoda T, Kojima T, Konagaya A, Suzuki H, Hayashizaki Y, Sakaki Y, Hatakeyama M. Integrative genome-wide expression analysis bears evidence of estrogen receptor-independent transcription in heregulin-stimulated MCF-7 cells. *PLoS ONE* 2008;3 (3):e1803. [PubMed: 18350142]
- Nephew KP, Long X, Osborne E, Burke KA, Ahluwalia A, Bigsby RM. Effect of estradiol on estrogen receptor expression in rat uterine cell types. *Biol Reprod* 2000;62 (1):168–177. [PubMed: 10611082]
- Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA. Mechanisms of estrogen action. *Physiol Rev* 2001;81 (4):1535–1565. [PubMed: 11581496]
- Oesterle E, Cunningham D, Westrum L, Rubel E. Ultrastructural analysis of [3H]thymidine-labeled cells in the rat utricular macula. *J Comp Neurol* 2003;463 (2):177–195. [PubMed: 12815755]

- Ogata Y, Slepecky NB, Takahashi M. Study of the gerbil utricular macula following treatment with gentamicin, by use of bromodeoxyuridine and calmodulin immunohistochemical labelling. *Hear Res* 1999;133 (1–2):53–60. [PubMed: 10416864]
- Ogawa S, Inoue S, Watanabe T, Orimo A, Hosoi T, Ouchi Y, Muramatsu M. Molecular cloning and characterization of human estrogen receptor betacx: a potential inhibitor of estrogen action in human. *Nucleic Acids Res* 1998;26 (15):3505–3512. [PubMed: 9671811]
- Ohnemus U, Uenal M, Inzunza J, Gustafsson JA, Paus R. The hair follicle as an estrogen target and source. *Endocr Rev* 2006;27 (6):677–706. [PubMed: 16877675]
- Okulicz G. Inner ear disturbance following long-term usage of hormonal contraceptives (author's transl). *HNO* 1978;26 (10):330–334. [PubMed: 711515]
- Osborne CK, Schiff R, Fuqua SA, Shou J. Estrogen receptor: current understanding of its activation and modulation. *Clin Cancer Res* 2001;7(12 Suppl):4338s–4342s. (discussion 4411s–4412s). [PubMed: 11916222]
- Oshima K, Grimm CM, Corrales CE, Senn P, Martinez Monedero R, Geleoc GS, Edge A, Holt JR, Heller S. Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. *J Assoc Res Otolaryngol* 2007;8 (1):18–31. [PubMed: 17171473]
- Paez-Pereda M, Giacomini D, Refojo D, Nagashima AC, Hopfner U, Grubler Y, Chervin A, Goldberg V, Goya R, Hentges ST, Low MJ, Holsboer F, Stalla GK, Arzt E. Involvement of bone morphogenetic protein 4 (BMP-4) in pituitary prolactinoma pathogenesis through a Smad/estrogen receptor crosstalk. *Proc Natl Acad Sci USA* 2003;100 (3):1034–1039. [PubMed: 12552124]
- Pak TR, Chung WC, Roberts JL, Handa RJ. Ligand-independent effects of estrogen receptor beta on mouse gonadotropin-releasing hormone promoter activity. *Endocrinology* 2006;147 (4):1924–1931. [PubMed: 16439454]
- Pappas TC, Gametchu B, Watson CS. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. *Faseb J* 1995;9 (5):404–410. [PubMed: 7896011]
- Picciotti PM, Fetoni AR, Paludetti G, Wolf FI, Torsello A, Troiani D, Ferraresi A, Pola R, Sergi B. Vascular endothelial growth factor (VEGF) expression in noise-induced hearing loss. *Hear Res* 2006;214 (1–2):76–83. [PubMed: 16603326]
- Pietras RJ, Arboleda J, Reese DM, Wongvipat N, Pegram MD, Ramos L, Gorman CM, Parker MG, Sliwkowski MX, Slamon DJ. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. *Oncogene* 1995;10 (12):2435–2446. [PubMed: 7784095]
- Pirskanen A, Kiefer JC, Hauschka SD. IGFs, insulin, Shh, bFGF, and TGF-beta1 interact synergistically to promote somite myogenesis in vitro. *Dev Biol* 2000;224 (2):189–203. [PubMed: 10926759]
- Pleis JR, Lethbridge-Cejku M. Summary health statistics for US adults: National Health Interview Survey, 2005. *Vital Health Stat* 2006;10 (232):1–153.
- Power RF, Mani SK, Codina J, Conneely OM, O'Malley BW. Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science* 1991;254 (5038):1636–1639. [PubMed: 1749936]
- Presson JC, Popper AN. Possible precursors to new hair cells, support cells, and Schwann cells in the ear of a post-embryonic fish. *Hear Res* 1990;46 (1–2):9–21. [PubMed: 2380129]
- Ramsey TL, Risinger KE, Jernigan SC, Mattingly KA, Klinge CM. Estrogen receptor beta isoforms exhibit differences in ligand-activated transcriptional activity in an estrogen response element sequence-dependent manner. *Endocrinology* 2004;145 (1):149–160. [PubMed: 14500565]
- Raphael Y, Adler HJ, Wang Y, Finger PA. Cell cycle of transdifferentiating supporting cells in the basilar papilla. *Hear Res* 1994;80 (1):53–63. [PubMed: 7852203]
- Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* 2005;307 (5715):1625–1630. [PubMed: 15705806]
- Riese DJ 2nd, Stern DF. Specificity within the EGF family/ErbB receptor family signaling network. *Bioessays* 1998;20 (1):41–48. [PubMed: 9504046]
- Roberson D, Rubel E. Cell division in the gerbil cochlea after acoustic trauma. *Am J Otol* 1994;15 (1):28–34. [PubMed: 8109626]

- Roberson DW, Alosi JA, Cotanche DA. Direct transdifferentiation gives rise to the earliest new hair cells in regenerating avian auditory epithelium. *J Neurosci Res* 2004;78 (4):461–471. [PubMed: 15372572]
- Roepke TA, Xue C, Bosch MA, Scanlan TS, Kelly MJ, Ronnekleiv OK. Genes Associated with Membrane-initiated Signaling of Estrogen and Energy Homeostasis. *Endocrinology* 2008;149 (12): 6113–6124. [PubMed: 18755790]
- Rubel E, Dew L, Roberson D. Mammalian vestibular hair cell regeneration. *Science* 1995;267 (5198): 701–707. [PubMed: 7839150]
- Ruben RJ. Development of the inner ear of the mouse: a radioautographic study of terminal mitoses. *Acta Otolaryngol: Suppl* 1967;220:1–44.
- Ryals B, Rubel E. Hair cell regeneration after acoustic trauma in adult Coturnix quail. *Science* 1988;240 (4860):1774–1776. [PubMed: 3381101]
- Sajan SA, Warchol ME, Lovett M. Toward a systems biology of mouse inner ear organogenesis: gene expression pathways, patterns and network analysis. *Genetics* 2007;177 (1):631–653. [PubMed: 17660535]
- Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP, Meng YG, Ferrara N, Jaffe RB, Taylor RN. Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. *J Clin Endocrinol Metab* 1996;81 (8):3112–3118. [PubMed: 8768883]
- Sinkevicius KW, Burdette JE, Woloszyn K, Hewitt SC, Hamilton K, Sugg SL, Temple KA, Wondisford FE, Korach KS, Woodruff TK, Greene GL. An estrogen receptor- α knock-in mutation provides evidence of ligand-independent signaling and allows modulation of ligand-induced pathways in vivo. *Endocrinology* 2008;149 (6):2970–2979. [PubMed: 18339713]
- Sobkowicz HM, August BK, Slapnick SM. Epithelial repair following mechanical injury of the developing organ of Corti in culture: an electron microscopic and autoradiographic study. *Exp Neurol* 1992;115 (1):44–49. [PubMed: 1728572]
- Sobkowicz HM, August BK, Slapnick SM. Post-traumatic survival and recovery of the auditory sensory cells in culture. *Acta Otolaryngol* 1996;116 (2):257–262. [PubMed: 8725527]
- Sobkowicz HM, August BK, Slapnick SM. Cellular interactions as a response to injury in the organ of Corti in culture. *Int J Dev Neurosci* 1997;15 (4–5):463–485. [PubMed: 9263026]
- Son E, Lee J, Lee S, Kim M, Lee B, Chang I, Chung J. Topical application of 17 β -estradiol increases extracellular matrix protein synthesis by stimulating *tgf*- β signaling in aged human skin in vivo. *J Invest Dermatol* 2005;124 (6):1149–1161. [PubMed: 15955089]
- Stenberg A, Wang H, Sahlin L, Hultcrantz M. Mapping of estrogen receptors α and β in the inner ear of mouse and rat. *Hear Res* 1999;136 (1–2):29–34. [PubMed: 10511621]
- Strachan D. Sudden sensorineural deafness and hormone replacement therapy. *J Laryngol Otol* 1996;110 (12):1148–1150. [PubMed: 9015429]
- Talwar RM, Wong BS, Svoboda K, Harper RP. Effects of estrogen on chondrocyte proliferation and collagen synthesis in skeletally mature articular cartilage. *J Oral Maxillofac Surg* 2006;64 (4):600–609. [PubMed: 16546639]
- Tanapat P, Hastings NB, Reeves AJ, Gould E. Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J Neurosci* 1999;19 (14):5792–5801. [PubMed: 10407020]
- Tang S, Tan SL, Ramadoss SK, Kumar AP, Tang MH, Bajic VB. Computational method for discovery of estrogen responsive genes. *Nucleic Acids Res* 2004;32 (21):6212–6217. [PubMed: 15576347]
- Teng J, Wang ZY, Prossnitz ER, Bjorling DE. The G protein-coupled receptor GPR30 inhibits human urothelial cell proliferation. *Endocrinology* 2008;149 (8):4024–4034. [PubMed: 18467434]
- Thomas P, Dong J. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. *J Steroid Biochem Mol Biol* 2006;102 (1–5):175–179. [PubMed: 17088055]
- Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* 2005;146 (2):624–632. [PubMed: 15539556]

- Vyhlidal C, Samudio I, Kladde MP, Safe S. Transcriptional activation of transforming growth factor alpha by estradiol: requirement for both a GC-rich site and an estrogen response element half-site. *J Mol Endocrinol* 2000;24 (3):329–338. [PubMed: 10828826]
- Wang C, Prossnitz ER, Roy SK. G protein-coupled receptor 30 expression is required for estrogen stimulation of primordial follicle formation in the hamster ovary. *Endocrinology* 2008;149 (9):4452–4461. [PubMed: 18499747]
- Warchol M, Lambert P, Goldstein B, Forge A, Corwin J. Regenerative proliferation in inner ear sensory epithelia from adult guinea pigs and humans. *Science* 1993;259 (5101):1619–1622. [PubMed: 8456285]
- Watson CS, Alyea RA, Hawkins BE, Thomas ML, Cunningham KA, Jakubas AA. Estradiol effects on the dopamine transporter – protein levels, subcellular location, and function. *J Mol Signal* 2006;1:5. [PubMed: 17224081]
- Watson CS, Jeng YJ, Kochukov MY. Nongenomic actions of estradiol compared with estrone and estriol in pituitary tumor cell signaling and proliferation. *Faseb J* 2008;22 (9):3328–3336. [PubMed: 18541692]
- Weigel NL, Zhang Y. Ligand-independent activation of steroid hormone receptors. *J Mol Med* 1998;76 (7):469–479. [PubMed: 9660165]
- Xu L. Regulation of Smad activities. *Biochim Biophys Acta* 2006;1759 (11–12):503–513. [PubMed: 17182123]
- Yamamoto T, Saatcioglu F, Matsuda T. Cross-talk between bone morphogenic proteins and estrogen receptor signaling. *Endocrinology* 2002;143 (7):2635–2642. [PubMed: 12072396]
- Yamashita H, Oesterle E. Induction of cell proliferation in mammalian inner-ear sensory epithelia by transforming growth factor alpha and epidermal growth factor. *Proc Natl Acad Sci USA* 1995;92 (8):3152–3155. [PubMed: 7724532]
- Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2001;2 (2):127–137. [PubMed: 11252954]
- Zhang M, Yan JD, Zhang L, Wang Q, Lu SJ, Zhang J, Zhu TH. Activation of bone morphogenetic protein-6 gene transcription in MCF-7 cells by estrogen. *Chin Med J (Engl)* 2005;118 (19):1629–1636. [PubMed: 16232348]
- Zheng JL, Frantz G, Lewis AK, Sliwkowski M, Gao WQ. Heregulin enhances regenerative proliferation in postnatal rat utricular sensory epithelium after ototoxic damage. *J Neurocytol* 1999;28 (10–11):901–912. [PubMed: 10900093]
- Zheng JL, Gao WQ. Analysis of rat vestibular hair cell development and regeneration using calretinin as an early marker. *J Neurosci* 1997;17 (21):8270–8282. [PubMed: 9334402]
- Zheng JL, Helbig C, Gao WQ. Induction of cell proliferation by fibroblast and insulin-like growth factors in pure rat inner ear epithelial cell cultures. *J Neurosci* 1997;17 (1):216–226. [PubMed: 8987750]
- Zhou S, Turgeman G, Harris SE, Leitman DC, Komm BS, Bodine PV, Gazit D. Estrogens activate bone morphogenetic protein-2 gene transcription in mouse mesenchymal stem cells. *Mol Endocrinol* 2003;17 (1):56–66. [PubMed: 12511606]
- Zine A, de Ribaupierre F. Replacement of mammalian auditory hair cells. *Neuroreport* 1998;9 (2):263–268. [PubMed: 9507966]

Fig. 1.

Schematic representation of the molecular mechanisms of estrogen receptors. The classical pathway of estrogen signaling is ligand-dependent activation of estrogen receptors (ERs) and direct genomic effects on gene regulation by binding estrogen response elements (ERE). The ligand-dependent indirect genomic regulation of gene transcription includes interaction with other transcription factors (TF). In ligand-dependent signaling the ligand activates either membrane-associated classical ERs and/or the G-protein-coupled receptor, GPR30, to initiate signaling cascades via second messengers. This leads to rapid physiological response without involving gene regulation. The ligand-independent pathway can be stimulated by other signaling pathways, such as growth factor signaling, and elicits both genomic and non-genomic effects. The activated kinases phosphorylate estrogen receptors, initiating dimerization and regulation of gene transcription or activation of second messengers. (Adapted from Heldring et al., 2007).

Table 1

EGF signaling family.

Ligands	Receptors	Signaling proteins
EGF	ErbB1	Akt
TGF α	ErbB2*	GAP/Ras
HB-EGF	ErbB3 [#]	HB-EGF
Amphiregulin	ErbB4	Jak
β -cellulin		PKC
Epiregulin		PLC γ
NRG1		Shp2
Glial growth factor (GGF2)		STAT
Heregulin (HRG)		PAK-JNKK-JNK
SMDF		RAF-MEK-MAPK
ARIA		
NDF		
NRG2		
NRG3		
NRG4		

Ligands: List of known ErbB ligands. Receptors: ErbB1 (EGFR, HER1), ErbB2 (neu or HER2),

* binds no ligand with high affinity, ErbB3 (HER3),

[#] inactive catalytic domain, and ErbB4 (HER4). Signaling proteins: Only some of the pathways and transcription factors are represented in this table.

Abbreviations: Epidermal growth factor (EGF), Transforming growth factor- α , (TGF α), Heparin-binding EGF (HB-EGF), Neuregulin (NRG), Sensory and motor neuron-derived factor (SMDF), Acetylcholine receptor inducing activity (ARIA), and neu-differentiation factor (NDF). Akt, a serine/threonine kinase; GAP, GTPase activating protein; HB-EGF, heparin-binding EGF; Jak, janus kinase; PKC, protein kinase C; PLC γ , phospholipase C γ , Shp2; Src homology domain-2-containing protein tyrosine phosphatase 2; STAT, signal transducer and activator of transcription; RAF-MEK-MAPK and PAK-JNKK-JNK, serine/threonine kinases. (Adapted from Yarden and Sliwkowski, 2001).

Table 2TGF β signaling superfamily.

Type II Rreceptor	Type 1 receptor	Ligands	R-SMAD
TGF β RII	TGF β RI/ALK5	TGF β 1	SMAD1,5,8
	Acvr1/ALK1	TGF β 2	or
	Acvr1/ALK2	TGF β 3	SMAD2,3
Acvr2a		ACTIVIN- β A	
	Acvr1b/ALK4	ACTIVIN- β A,B,C,E	SMAD2,3
	Acvr1c/ALK7	BMP2	or
	BMPRI/ALK6	BMP6	SMAD1,5,8
	BMPRI/ALK3	BMP7	
		GDF5	
		GDF8	
		GDF9	
		GDF11	
		NODAL	
Acvr2b	Acvr1/ALK2	ACTIVIN- β A,B,C,E	SMAD2,3
	Acvr1b/ALK4	NODAL	
	Acvr1c/ALK7	BMP2	
		BMP6	
		BMP7	
BMPRII	Acvr1/ALK2	BMP2	SMAD1,5,8
	BMPRI/ALK3	BMP4	
	BMPRI/ALKE	BMP6	
		BMP7	
		GDF5	
		GDF6	
AMHR		GDF9	
	Acvr1/ALK2	MIS	SMAD1,5,8
	BMPRI/ALK3		
	BMPRI/ALKB		

Type II Receptors: There are five Type II receptors; Transforming growth factor beta receptor (TGF β RII), Activin receptor A (Acvr2a), Activin receptor B (Acvr2b), Bone morphogenic protein receptor (BMPRII) and Anti-Mullerian hormone receptor (AMHR). Type I receptors are also called the Activin-like kinases (ALKs) and listed according to known dimerizations with type II receptors. Ligands that correspond to Type I and Type II receptor dimer binding are listed, however, not all of the TGF β ligands are listed. Mullerian inhibitory substance (MIS), growth differentiation factor (GDF). SMAD signaling pathway associated with each receptor/ligand interaction is shown. Some receptor dimers can signal through either SMAD pathway depending on the activating ligand.