their actions by activating nuclear receptors, transcription factors that regulate gene expression. The glucocorticoid receptor (GR) is the transcription factor that predominantly mediates both physiological and pharmacological glucocorticoid effects. Yet glucocorticoids can also bind and activate the mineralocorticoid receptor (MR), a transcription factor known to bind aldosterone thus maintaining whole-body fluid homeostasis. Phylogenetically, GR and MR are closely related and share a remarkable structural similarity. Indeed, the DNA-binding domain of MR is 96% identical to that of GR; thus MR is recruited to many of the same DNA response elements that bind GR. Moreover, GR has a low affinity for glucocorticoids but is expressed in nearly every cell, whereas MR shows a higher affinity for glucocorticoids although knowledge of MR's expression levels is somewhat limited. These characteristics suggest that, while GR and MR can compensate for each other's actions in many tissues, there are specific glucocorticoid and mineralocorticoid-mediated responses indicating GR-MR functional diversity. To investigate the similarities and differences between GR and MR signaling in the presence of glucocorticoid hormones, we generated U-2 OS (human osteosarcoma) cell lines stably expressing GR, MR, and both GR and MR (MRGR). Immunofluorescence analysis showed that the treatment of these cell lines with 1 nM of the synthetic glucocorticoid dexamethasone (Dex) induced nuclear translocation of both GR and MR. Moreover, Proximity Ligation Assay revealed that, in the absence of ligand, GR associated with MR in the cytoplasm and, upon 1 nM Dex exposure, GR-MR complexes were detected in the nucleus of MRGR cells. To decipher the functional contribution of GR-MR complexes in the transcriptional response to Dex, we performed RNA-seq in GR, MR, and MRGR cells treated with 1 nM of Dex. Transcriptome analysis revealed that Dex-activated GR regulated the transcription of 6180 genes. Co-expression of MR resulted in a greatly blunted Dex-mediated gene response which reduced the glucocorticoid-dependent transcriptome size by 75%. This phenomenon was also observed using a higher concentration of Dex. Indeed, 40% of genes commonly regulated by Dex in GR and MRGR cells showed a reduced magnitude of regulation when MR is co-expressed. These results suggest a functional antagonism between GR and MR in which MR inhibits GR function. Understanding the molecular mechanisms governing the cross-talk between GR and MR is crucial for the development of new therapies that address the adverse effects of glucocorticoid treatment as well as for the discovery of novel glucocorticoid-based therapeutics with minimal side effects.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Optimized Immunohistochemical Detection of Rat ESR2 Proteins Using the Specific Anti-ESR2 Monoclonal Antibody PPZ0506

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Background: Research on ESR2, also known as estrogen receptor β (ERβ), is a notorious example of data distortion due to the use of inadequately validated antibodies. Although the absence of reliable specific antibodies against ESR2 has severely hindered the promotion of ESR2 research, a specific anti-human ESR2 monoclonal antibody (PPZ0506) was identified in 2017 [1]. Our previous study confirmed its cross-reactivity and specificity against rodent ESR2 proteins, enabling the elucidation of the true ESR2 distribution in rodents [2].
Objective: We aimed to determine the optimized conditions for immunohistochemical detection of rat ESR2 proteins using PPZ0506. <Method> Several staining conditions using paraffin-embedded and frozen ovary sections were evaluated, and the distribution of rat ESR2 proteins was analyzed under optimal conditions.
Result: Immunohistochemical staining with PPZ0506 required appropriate antigen retrieval and antibody dilution. Subsequent immunohistochemical analysis in multiple tissues under optimized conditions revealed that rat ESR2 proteins are expressed in a more localized manner than previously assumed. Our optimized immunohistochemical detection of rat ESR2 proteins, using a well-validated antibody, revealed their distribution in limited tissues and cell types.
Conclusion: Our results suggest that previous immunohistochemical studies using inadequately validated antibodies against ESR2 proteins overestimated their distribution profiles. We expect that our optimized immunohistochemistry using the PPZ0506 antibody may solve conflicting problems in ESR2 research.
References: 1. Andersson S, *et al.* Nat Commun 15;8:15840 (2017) 2. Ishii H, *et al.* Int J Mol Sci 20(24):6312 (2019)

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Paxillin Is Required for Androgen Receptor Expression in Granulosa Cells

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Androgens are important in female reproduction, as evident from studies of mouse ovary-specific androgen receptor knockout models characterized by sub-fertility and diminished ovarian reserve. Androgen activity specifically promotes granulosa cell proliferation and follicle progression. However, the molecular mechanisms mediating androgen activity in granulosa cells are unknown. Our lab previously showed that the cytoplasmic adaptor protein paxillin is required for full transcriptional activity by the androgen receptor (AR) in prostate cancer cells, therefore we examined how paxillin affects the androgen receptor in granulosa cells. We found that paxillin knockdown results in significantly reduced AR protein levels, independently of *AR* gene transcription in human granulosa-derived KGN cells. Similar to previous data from other cell types, we found that paxillin directly interacts with poly-A binding protein (PABP) in KGN cells using proximity ligation assay. Ligand binding further increases AR protein expression by reducing its degradation. Using immunofluorescence, we