Chapter 15
Androgen Receptor Mutations Associated with Androgen Insensitivity Syndrome: A High Content Analysis Approach Leading to Personalized Medicine

Adam T. Szafran, Huiying Sun, Sean Hartig, Yuqing Shen, Sanjay N. Mediwala, Jennifer Bell, Michael J. McPhaul, Michael A. Mancini, and Marco Marcelli

Androgen insensitivity syndrome (AIS) is a rare disease associated with inactivating mutations of AR that disrupt male sexual differentiation and cause a spectrum of phenotypic abnormalities having as a common denominator loss of reproductive viability. No established treatment exists for this condition; however, there are sporadic reports of patients (or recapitulated mutations in cell lines) that respond to administration of supraphysiologic doses (or pulses) of testosterone or synthetic ligands. The common denominator of these mutations is that they are located in the ligand-binding domain (LBD) and are associated with qualitative abnormal ^{3}H-DHT binding consisting of increased ligand–receptor dissociation rate.

We have utilized a novel high content analysis (HCA) approach to study AR function at the single cell level in genital skin fibroblasts (GSF) from patients and in HeLa cells stably transfected with plasmids containing wtAR or the mutation of interest fused to a green fluorescent protein (GFP) [1]. We have completed AR HCA analysis in three patients with AIS. While patients with mutations F764L and P766S were affected by complete androgen insensitivity syndrome (CAIS), mutation R840C was found in a patient affected by partial androgen insensitivity (PAIS). The biochemical phenotype of receptor mutants F764L and P766S consisted in normal K_d and B_{max} and increased ligand dissociation rate, while that of mutant R840C consisted in normal K_d, B_{max}, and ligand dissociation rate, but presence of thermolability (that is, the binding ability of this receptor decreased to more than 50% when the temperature was supraphysiologic). All three mutant receptors were greatly

M. Marcelli (✉)
Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA; Department of Medicine, Baylor College of Medicine, Houston, TX 77030, USA; Baylor College of Medicine, Michael E. DeBakey VA Medical Center, Houston, TX 77030, USA
e-mail: marcelli@bcm.edu

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impaired in assays of transcriptional activity. AR HCA provides an opportunity to simultaneously quantify a myriad of cellular conditions associated with AR functions, is fully amendable to high throughput rates of data collection and automated analyses, and permits us to quantify AR activities in response to ligands, mutations, and various perturbations of the intracellular environment of the host cell and to identify anti-androgens with novel mechanism of action. With data based upon well-defined selection criteria from hundreds of cells per condition generated by the software PLP (Pipeline Pilot, Acelrys, San Diego, CA, USA) and CyteSeer (Vala Sciences, San Diego, CA, USA) we are able to define the ligand dose necessary to induce or inhibit (EC_{50}/IC_{50}) five sequential features that contribute to AR activation: (1) total AR protein level; (2) percent localization of signal in the nucleus (e.g., the nuclear:cytoplasmic ratio); (3) formation of AR-rich subnuclear "speckles" that correlate with AR transcriptional activity; (4) transcriptional reporter gene activity; and (5) AR nuclear export.

For our studies we used HeLa cells transfected with the GFP-fused plasmid of interest and treated with logarithmic concentrations of DHT, mibolerone, or R1881. GSF from each patient and six normal controls received the same experimental treatment, while their endogenous AR was visualized by antibody staining. AR HCA was able to provide for HeLa cells data on the percent localization of the signal in the nucleus, the formation of AR-rich subnuclear speckles, and the transcriptional reporter gene activity. In contrast to GSF we were able to obtain data only on the percent localization of the signal in the nucleus and on the formation of AR-rich subnuclear speckles. Reporter gene transcriptional activity was not obtained because no AR-responsive promoter could be found that works in GSF.

The measured functions of P766S activity could be rescued at EC_{50} 10-fold higher compared to wild-type AR in both HeLa and GSF using each of the three ligands. F764L functions were rescued, but only in a mibolerone-dependent way and at EC_{50} concentrations 10- to 30-fold higher than wtAR, while R840C could not be rescued under any experimental condition. We hypothesized that the rescue of AR functions was associated with an increased level of ligand–receptor complex stability. To prove this we performed a two-hybrid analysis determining the AR NH_2–COOH-terminal domain interaction, an assay measuring the tightness of receptor–ligand binding. In agreement with the HCA data, these experiments showed that the degree of AR NH_2–COOH interaction increased as a function of ligand concentration for all three agonists using P766S. For F764L, AR NH_2–COOH interaction increased only as a function of mibolerone concentrations, while for R840C it did not change at all under any experimental conditions.

The conclusions of this study are that certain LBD AIS mutations associated with normal K_d and B_{max} but abnormal ligand dissociation rate can be rescued by AR agonists given at supraphysiologic concentrations and that higher concentration of agonist works by increasing the ligand–receptor complex stability. In addition, these experiments support the notion that HCA can be used for personalized treatment of patients affected by AIS.

References

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