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Drug transporters in spermatogenesis

A re-evaluation of recent data on P-glycoprotein

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Abbreviations: ABC, ATP-binding cassette; BBB, blood-brain barrier; BTB, blood-testis barrier; ERM, ezrin, radixin and moesin; FAK, focal adhesion kinase; JAM-A, junctional adhesion molecule-A; MDR, multidrug resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; OATP3, organic anion transporter protein 3; RNAi, RNA interference; SLC, solute carrier; TJ, tight junction; ZO-1, zonula occludens-1

Drug transporters are integral membrane proteins expressed by a variety of organs, including the liver, kidney, small intestine and testis, and they are generally known to mediate drug or xenobiotic transport into and out of cells. Previous studies have also reported the presence of several drug transporters at blood-tissue barriers where they are thought to protect organs from harmful agents. In this editorial, we briefly discuss and re-evaluate recent findings that show P-glycoprotein, an efflux pump, to function at the blood-testis barrier. We also put forth a mechanistic model, hoping this information will form a strong basis for future studies.

Multidrug resistance (MDR) occurs when a disease-causing organism (i.e., bacteria, fungi or parasite) or a disease-causing cell (i.e., neoplastic or virally-infected) does not respond to a pharmacological agent(s) that is designed to destroy it. One of the most well-known examples of MDR to make headlines in recent years relates to methicillin-resistant *Staphylococcus aureus* (MRSA), the so-called 'super bug' residing in many hospitals and nursing homes that is almost impossible to eradicate by trusted and widely prescribed antibiotics such as methicillin, amoxicillin and penicillin. MDR can also manifest itself in neoplastic cells, as well as in immune cells harboring, for example, the human immunodeficiency virus (HIV). In all cases, MDR is a condition that prevents a drug(s) from reaching or accumulating its therapeutic dose and results in drug insensitivity so that individuals presenting for MDR remain 'clinically untreated', and this can result in death. Because MDR limits treatment options for those with life-debilitating and life-threatening conditions, it is a major concern that continues to elude physicians and healthcare professionals, as well as researchers trying to find an effective way to reverse it.

It is not completely understood why the MDR phenotype is triggered in some individuals and not in others. While MDR is known to associate with several mechanisms, perhaps the most well studied involves drug transporters (i.e., increased expression of efflux pumps and/or decreased expression of influx pumps). Drug transporters are integral membrane proteins that pump drugs and xenobiotics, as well as endogenous compounds such as steroids, into or out of cells, and two superfamilies have been identified in mammals: the ATP-binding cassette (ABC) superfamily of efflux transporters (P-glycoprotein or MDR1 is the best-studied efflux pump) and the solute carrier (SLC) superfamily of influx transporters.[1-4] Interestingly, drug transporters are not only expressed by disease-causing organisms and cells;[5-10] normal cells such as hepatocytes, epithelial cells in the intestine, Sertoli and germ cells in the testis are also known to express drug transporters. In fact, many drug transporters are known to be present at blood-tissue barriers where they are presumed to protect sensitive organs from dangerous exogenous agents.[11-15] For example, knockout of *Mdr1a* (in rodents, MDR1 is encoded by *Mdr1a* and *Mdr1b*), which was previously shown to localize at the blood-brain (BBB) and the blood-testis barrier (BTB), rendered mice more susceptible to the harmful effects of ivermectin (an antiparasitic drug) and vinblastine (an anticancer drug).[16] Specifically, increased uptake of these compounds was observed in the brain and in the testis of *Mdr1a*$^{-/-}$ vs. *wt* mice, illustrating that P-glycoprotein safeguards these organs by pumping out or prohibiting entry of harmful substances.

Recent studies from our group, as well as those from other laboratories, have shown testicular cells to express an array of drug transporters. For example, P-glycoprotein localized to Sertoli cells, late spermatids, Leydig cells and peritubular myoid cells.[17,18] Within Sertoli cells, P-glycoprotein, as well as another drug transporter [i.e., organic anion transporter protein 3 (OATP3)], concentrated to the site of the BTB, and P-glycoprotein co-immunoprecipitated with structural tight junction (TJ) proteins, namely occludin, claudin-11 and junctional adhesion molecule-A

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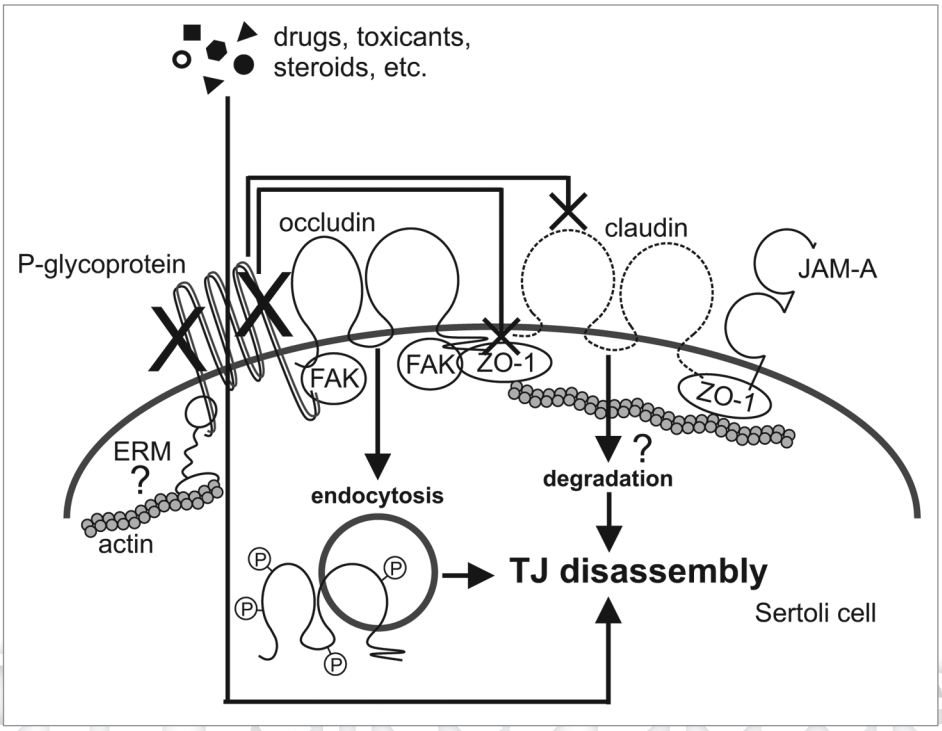

Figure 1. A mechanistic model of P-glycoprotein function at the BTB. Previous studies have shown P-glycoprotein to localize at the BTB and to co-immunoprecipitate with structural TJ proteins, namely occludin, claudin-11 and JAM-A, which assemble into a multi-protein complex via ZO-1 and FAK (see text for references). To better understand the role of P-glycoprotein at the BTB, Sertoli cells were isolated from testes of 20-d-old rats and cultured at high density on Matrigel-coated substrata for 3–4 d to allow the assembly of a functional Sertoli cell barrier. Thereafter, *Mdr1* was silenced by RNAi (denoted by an "X"), and barrier function was assessed by transepithelial electrical resistance measurements, immunoblotting and immunolocalization experiments. Following knockdown, Sertoli cell barrier dysfunction was noted. Specifically, the association between occludin and ZO-1 was disrupted (denoted by connected lines that end with an "X"), which increased the association between occludin and FAK. This resulted in the Thr phosphorylation (denoted by an encircled "P") and in the endocytosis (denoted by a large circle) of occludin, disrupting the integrity of TJs. A decrease in the steady-state level of claudin-11 (denoted by a dotted-line and by connected lines that end with an "X") was also observed following knockdown, contributing to TJ dysfunction as well. BTB dysfunction resulting from the loss of P-glycoprotein may have also allowed entry of drugs, toxicants and steroids (denoted by an assortment of symbols), which would have otherwise been prohibited from entering the adluminal compartment of the seminiferous epithelium or pumped out in the presence of P-glycoprotein at the BTB. An additional study has reported P-glycoprotein to associate with actin via the ezrin-radixin and moesin (ERM) protein complex,[28] but it is not known if this finding applies to the testis as well. Future experiments are also needed to identify the mechanism behind the decrease in claudin-11 following knockdown of *Mdr1*. For simplicity, other junction types that contribute to BTB function have been omitted, and only selected TJ proteins have been included.

(JAM-A), when normal testis and/or Sertoli cell lysates were used.[18-20] Equally important, knockdown of *Mdr1a* + *Mdr1b* (referred to as *Mdr1* from here onwards) in rat Sertoli cells by RNA interference (RNAi) was found to partially compromise the integrity of the Sertoli cell barrier when its function was assessed by transepithelial electrical resistance measurements, as well as by immunoblotting and immunolocalization experiments.[19] Interestingly, destabilization of the Sertoli cell barrier following *Mdr1* RNAi appeared to involve multiple mechanisms that may or may not be inter-related (**Fig. 1**). Following *Mdr1* silencing, occludin was found to dissociate from zonula occludens-1 (ZO-1) and to associate increasingly with focal adhesion kinase (FAK), which brought about changes in occludin phosphorylation (i.e., a decrease and an increase in overall Ser and Thr phosphorylation, respectively, with no changes in overall Tyr phosphorylation).[19] However, the significance of these results is not immediately known because in general hyper-phosphorylation of Ser-/Thr-occludin associates with TJ stability, whereas hyper-phosphorylation of Tyr-occludin associates with TJ dysfunction.[21,22] These changes may be related to the uniqueness of the BTB, and future experiments employing site-specific phospho antibodies against occludin are likely to be more informative. Nevertheless, these results still fall in line with those obtained from endocytosis assays, which demonstrated an increase in occludin internalization over control levels[19] (**Fig. 1**). Another mechanism relates to the decline (albeit moderate) in the steady-state level of claudin-11 that was noted following *Mdr1* RNAi, but at this point we cannot conclude if this decrease in claudin-11 was the result of increased protein degradation or decreased protein synthesis. Certainly, mislocalization of occludin may have provoked the mislocalization of claudin-11 away from the plasma membrane, contributed to its instability and triggered its degradation. Other mechanisms are likely to be at play as well. For instance, *Mdr1* silencing may have allowed entry of unknown compounds past the BTB and into the adluminal compartment of Sertoli cells, which would have otherwise been prohibited from

entering or pumped out in the presence of P-glycoprotein at the BTB. This may have also affected BTB function (**Fig. 1**). At this point, additional studies are needed to better understand the role of P-glycoprotein and other drug transporters at the BTB.

What might some of these studies be? First, would a disruption (or at least a partial disruption) of the Sertoli cell barrier/BTB affect P-glycoprotein-mediated drug efflux? For instance, would knockdown of a critical TJ protein such as claudin-11, which is known to bind P-glycoprotein, increase the influx of P-glycoprotein substrates? If yes, one could conclude that BTB integrity is essential for P-glycoprotein function. Second, is aberrant regulation of drug transporters (efflux and influx pumps alike) linked to unexplained cases of male infertility? Environmental toxicants can utilize drug transporters expressed by the testis. The best example of this is the entry of cadmium by SLC39A8, a xenobiotic transporter and influx pump.[23,24] Thus, targeted manipulation of drug transporter expression may protect the testis from harmful substances and preserve fertility. This may also be applicable to men receiving chemotherapy, which in many cases is known to jeopardize their ability to father children. Finally, internalization of P-glycoprotein has also been reported, and when this occurs, P-glycoprotein function is compromised.[25-27] In-depth investigations along these fronts are likely to yield interesting observations, not only with respect to P-glycoprotein function but also with respect to BTB dynamics which are critical for spermatogenesis.

Disclosure of Potential Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported herein.

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