Protein kinases regulate hyperactivated motility of human sperm

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Once the sperm enters the female reproductive tract, they undergo an important process called capacitation, which involves a series of physiological and biochemical changes that ensure fertilization. During capacitation, calcium ions (Ca²⁺) increase the bending amplitude of a sperm’s flagellum. This causes hyperactivation, which is manifested as the asymmetrical beating of the flagellum. Tyrosine phosphorylation is also a cause for human sperm hyperactivation; asthenospermia—that is, reduced sperm motility—occurs when the sperm are incapable of hyperactivation owing to impaired tyrosine phosphorylation.[1] Hyperactivated motility enables the sperm to migrate and penetrate the cumulus cells and zona pellucida surrounding the oocyte, leading to fertilization. Therefore, the hyperactivated motility of sperm is critical for male reproductive ability, and the molecular mechanism underlying human sperm hyperactivation requires discussion.

Transcription and translation do not occur in sperm because they have highly compacted DNA and lack endoplasmic reticulum. Therefore, hyperactivated motility is primarily regulated by post-translational modifications, such as protein phosphorylation, which can activate or inhibit specific signaling pathways. Multiple protein kinases have been found in human sperm. These include protein kinase A (PKA), protein kinase B (also called AKT), protein kinase G (PKG), and mitogen-activated protein kinases (MAPKs), which play important roles in sperm motility.

Therefore, this medical progress article focuses on the roles of protein kinase phosphorylation in hyperactivated sperm motility. It will provide a better understanding of the signaling pathways involved and facilitate the discovery of new therapeutic targets for male infertility.

Recently, Wu et al[2] reported that C-type natriuretic peptide (CNP) secreted by the female genital tract improves human sperm motility. The researchers found that PKG is activated by CNP and 8-Br-Cyclic guanosine monophosphate (8-Br-cGMP, a cGMP analog). They postulated that CNP combines with natriuretic peptide receptor B, which is expressed on the acrosomal region of the head and the front end of the tail in human spermatozoa and increases intracellular cGMP. Downstream, cGMP activates PKG, which promotes Ca²⁺ influx and protein tyrosine phosphorylation by unknown steps, ultimately resulting in sperm hyperactivation.

Luconi et al[3] discovered that HCO₃⁻ facilitates motility and hyperactivation in human spermatozoa by recruiting and activating PKA. It directly activates soluble adenylyl cyclase (sAC) and causes adenosine triphosphate (ATP) to cyclize into cyclic adenosine monophosphate (cAMP), which subsequently activates PKA. The activated PKA then phosphorylates the tyrosine groups in sperm A-kinase anchor protein 3 (AKAP3). AKAP3 is a major scaffold protein that constitutes the fibrous sheath of the sperm tail, which controls the movement of the sperm flagellum and regulates hyperactivation. Other researchers have verified that hyperactivated motility in human sperm depends on the sAC/cAMP/PKA signaling pathway. They proposed that PKA phosphorylates and activates sarcoma protein kinase (Src), which mediates the phosphorylation of tyrosine groups in proteins involved in hyperactivated sperm motility. They also found that hyperactivated motility does not occur when sAC is inhibited but recovers when 8-Br-cAMP (a cAMP analog) is added.[4] Therefore, sAC/cAMP/PKA activation is necessary for the phosphorylation of AKAP3 and Src, which enables human sperm hyperactivation.

Phosphatidylinositol 3-kinase (PI3K)/AKT can be detected in the entire midpiece of human capacitated spermatozoa.

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The role of PI3K/AKT in regulating the hyperactivated motility of human sperm has been speculated by Sagare-Patil et al.[5]. In their view, phosphorylation of AKT at both Thr 308 and Ser 473 in human capacitated sperm is calcium-dependent and can be prevented by a PI3K inhibitor. Therefore, the influx of Ca^{2+} induced by progesterone activates PI3K and converts phosphatidylinositol-4,5-bisphosphate into phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 then binds to 3-phosphoinositide-dependent protein kinase 1 (PDK1) and activates it. PDK1 and PDK2 phosphorylate AKT at Thr 308 and Ser 473, respectively, and fully activate AKT, increasing sperm motility and subsequent hyperactivation. However, this finding contradicts with that of a study by Luconi et al.[6] which proposed that PI3K negatively regulates human sperm motility and that the PI3K inhibitor LY294002 triggers sperm forward motility, because PI3K inhibition leads to increased intracellular cAMP levels and PKA-AKAP3 interaction.

MAPKs are key kinases that are involved in the regulation of cell growth, differentiation, and stress. Almog et al.[7] reported that extracellular signal-regulated protein kinases 1/2 (ERK1/2) and p38 MAPK, but not c-Jun N-terminal kinase 1/2, are primarily localized in the tails of human sperm. Protein kinase C (PKC) is also expressed in the tails of mature human sperm and activates downstream ERK. Interestingly, both ERK1/2 and p38 are involved in regulating sperm motility, but their roles are completely opposite. Active ERK1/2 stimulates, whereas phosphorylated p38 inhibits positive and hyperactivated movements. Similarly, Yu et al.[8] conducted in vitro experiments and western blot analysis to verify that an increase in p38 phosphorylation is one of the reasons for the reduced sperm motility caused by arachidonic acid. Almog et al.[7] also found that PKC-stimulated ERK phosphorylates Rho GTPase-activating protein 6 (ARHGAP6), which is a specific GTPase-activating protein of Rho A and a promoter of actin remodeling. Because Rho A is involved in cell movement, and actin remodeling involves hyperactivation and acrosome reaction, ERK-phosphorylated ARHGAP6 may participate in the hyperactivation of human sperm.

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is an important enzyme for maintaining

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**Figure 1:** Signaling pathways of human sperm hyperactivation. AKAP3: A-kinase anchor protein 3; AMP: Adenosine monophosphate; AMPK: AMP-activated protein kinase; ARHGAP6: Rho GTPase-activating protein 6; ATP: Adenosine triphosphate; Ca^{2+}: Calcium ions; CNP: C-type natriuretic peptide; ERK: Extracellular signal-regulated protein kinase; NPR-B: Natriuretic peptide receptor B; PDK1: 3-phosphoinositide-dependent protein kinase 1; PDK2: 3-phosphoinositide-dependent protein kinase 2; PI3K: Phosphatidylinositol 3-kinase; PIP2: Phosphatidylinositol-4,5-bisphosphate; PIP3: Phosphatidylinositol-3,4,5-trisphosphate; PKA: Protein kinase A; PKC: Protein kinase C; Src: Sarcoma protein kinase.
cellular energy homeostasis. Its phosphorylation is independent of Ca^{2+} but dependent on the AMP/ATP ratio. AMPK also plays a central role in regulating sperm motility. The drug metformin markedly increases the percentage of hyperactivated sperm without affecting sperm Ca^{2+} levels. It also increases the phosphorylation of AMPK in human sperm. The activation of AMPK by A769662 is similar to the effect of metformin on human sperm. Furthermore, the downstream signaling pathway by which AMPK affects sperm motility remains unclear.

This article reviews the progress of research into protein kinase signaling pathways in the regulation of human sperm hyperactivation. Sperm motility does not interfere with spermatogenesis and hormone secretion, and it is an ideal target for male contraception. Protein kinases have become the primary targets for new drug development. The identification of protein kinases that regulate capacitation could provide new strategies for contraception or the treatment of unexplained male infertility. Therefore, kinase cascades in human sperm are worthy of further study.

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Conflicts of interest
None.

References
1. Buffone MG, Calamera JC, Verstraeten SV, Doncel GF. Capacitation-associated protein tyrosine phosphorylation and membrane fluidity changes are impaired in the spermatozoa of asthenozoospermic patients. Reproduction 2005;129:697–705. doi: 10.1530/rep.1.00584.
2. Wu K, Mei C, Chen Y, Guo L, Yu Y, Huang D. C-type natriuretic peptide regulates sperm capacitation by the cGMP/PKG signalling pathway via Ca^{2+} influx and tyrosine phosphorylation. Reprod Biomed Online 2019;38:289–299. doi: 10.1016/j.rbmo.2018.11.025.
3. Luconi M, Porazzi I, Ferruzzi P, Marchiani S, Forti G, Baldi E. Tyrosine phosphorylation of the a kinase anchoring protein 3 (AKAP3) and soluble adenylate cyclase are involved in the increase of human sperm motility by bicarbonate. Biol Reprod 2005;72:22–32. doi: 10.1095/bioreprod.104.032490.
4. Shahar S, Wiser A, Ickowicz D, Lubart R, Shulman A, Breireith H. Light-mediated activation reveals a key role for protein kinase A and sarcoma protein kinase in the development of sperm hyper-activated motility. Hum Reprod 2011;26:2274–2282. doi: 10.1093/humrep/der232.
5. Sagare-Patil V, Vernekar M, Galvankar M, Modi D. Progesterone utilizes the PI3K-AKT pathway in human spermatozoa to regulate motility and hyperactivation but not acrosome reaction. Mol Cell Endocrinol 2013;374:82–91. doi: 10.1016/j.mce.2013.04.005.
6. Luconi M, Carlson V, Marra F, Ferruzzi P, Forti G, Baldi E. Increased phosphorylation of AKAP by inhibition of phosphatidylinositol 3-kinase enhances human sperm motility through tail recruitment of protein kinase A. J Cell Sci 2004;117:1235–1246. doi: 10.1242/jcs.00931.
7. Almog T, Lazar S, Reiss N, Etkovitz N, Milch E, Rahamim N, et al. Identification of extracellular signal-regulated kinase 1/2 and p38 MAPK as regulators of human sperm motility and acrosome reaction and as predictors of poor spermatozoan quality. J Biol Chem 2008;283:14479–14489. doi: 10.1074/jbc.M710492200.
8. Yu L, Yang X, Ma B, Ying H, Shang X, He B, et al. Abnormal arachidonic acid metabolic network may reduce sperm motility via P38 MAPK. Open Biol 2019;9:180091. doi: 10.1098/rsbl.2018.0091.
9. Yang Y, Chen H, Weng S, Pan T, Chen W, Wang F, et al. In vitro exposure to metformin activates human spermatozoa at therapeutically relevant concentrations. Andrology 2020;8:663–670. doi: 10.1111/andr.12755.
10. Calle-Guisado V, Hurtado de Llera A, Gonzalez-Fernandez I, Bragado MJ, Garcia-Marin LJ. Human sperm motility is down-regulated by the AMPK activator A769662. Andrology 2017;5:1131–1140. doi: 10.1111/andr.12423.