RESEARCH PLAN:

Many lung diseases are associated with chronic exposure to alveolar hypoxia, resulting in the development of pulmonary hypertension (PH), a debilitating disease with high mortality due to right heart failure. Despite extensive study, the cellular mechanisms responsible for the pathogenesis of hypoxic PH are poorly understood. Morphometric and functional studies revealed that the development of PH is associated with both contraction and structural remodeling of the small pulmonary arteries. The remodeling is characterized by thickening of the smooth muscle cell (SMC) layer, a consequence of pulmonary arterial SMC (PASMC) hypertrophy and hyperplasia, and extension of new muscle around previously nonmuscular precapillary arterioles due to migration of PASMCs down the vascular tree[1-5]. While significant progress has been made in identifying mechanisms regulating PASMC proliferation and contraction during hypoxia, the mechanisms controlling migration are relatively unexplored.

Migration occurs in several cell types and has important implications in physiological and pathophysiological processes, including immune responses, angiogenesis, wound healing and tumor growth[6-8]. With respect to vascular SMCs, migration is best characterized in coronary SMCs during atherogenesis and restenosis following angioplasty[9-15]. Despite numerous studies describing SMC migration, the mechanisms involved are still not well understood, perhaps involving changes in the cytoskeleton, actin polymerization and activity of mitogen activated protein kinase (MAPK), all of which may occur secondary to increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)[10,12,15-18]. Data regarding migration of PASMCs is limited to a handful of reports[17,19-24], with most of these focusing on describing the phenomenon rather than mechanisms.

Studies in animal models have shown that endothelin-1 (ET-1) plays a substantial role in mediating hypoxic pulmonary hypertention[25-33]. We have generated preliminary data indicating that ET-1 causes smooth muscle migration although we have not yet elucidated the mechanisms mediating this response. Of interest, we have found that that ET-1 increases $[Ca^{2+}]_i$ in PASMCs[34], perhaps providing a clue to the pathway.

Recently, a new candidate has emerged as a regulator of cell migration: aquaporins (AQPs). AQPs are a family of proteins that form transmembrane channels which facilitate the transport of water into and out of cells[35,36]. The best characterized function of AQPs is in transepithelial permeability. Evidence now suggests that aquaporin 1 (AQP1) may also regulate migration of endothelial and tumor cells[35,37]. We found that PASMCs express AQP1; however, whether AQP1 plays a role in PASMC migration is unknown. Moreover, the factors controlling AQP1 expression/localization are not well explored, and while a rise in $[Ca^{2+}]_i$ results in altered expression/localization of other channels[38,39] and aquaporins[40,41], whether this occurs with AQP1 remains to be tested. The mechanism by which AQP1 meditates migration is not clear, although the prevailing hypothesis proposes that AQP1 facilitates localized water entry into lamellipodia [35,37,42]. We propose the alternate possibility that the cytoplasmic domain of AQP1 acts as a scaffold for cytoskeletal tethering, anchoring actin filaments to the cell membrane. Our preliminary data shows that actin filaments form in migrating cells and that AQP1 binds actin. Thus, we <u>hypothesize</u> that in PASMCs, increased $[Ca^{2+}]_i$ in response to ET-1 causes AQP1 to increase and/or redistribute within the cell, facilitating migration via actin binding and cytoskeletal rearrangement. To test this hypothesis we propose two Specific Aims:

Objective 1: **To determine the effect of ET-1 on AQP1 expression.**

Objective 2: To determine if AQP1-actin interaction is required for ET-1 induced migration.