Combating Diseases of Multi-Receptor Etiology by High-throughput Structure-Guided Targeting of a Unique Hub within Multi-Receptor Signal Networks

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Technology description

The inventors vision for the future of cancer therapy is successful identification and annihilation of the "sentinel node" of the entire metastasis network, not just individual hubs or one receptor at a time. Cancer invasion is multi-receptor in etiology; signals that drive tumor cell growth/invasion are initiated by a myriad of receptors on cell surface. While receptor-dependent signaling is transient due to receptor downregulation, desensitization, internalization and/or degradation, the existence of a subsequent receptor-independent step of signal enhancement (perpetuation and amplification by several folds) has long been recognized as an absolute pre-requisite to execute the cellular process. The origin of this essential step of amplification has remained an enigma. Consequently, the largest fraction of marketed anti-cancer agents is directed towards blocking the surface receptors/or individual pathways, while the second wave of amplification has remained unexploited. UCSD researchers have discovered such a powerful molecular interface, asentinel nodethat is positioned between convergent signaling pathways downstream of a variety of surface receptors, which enhances prometastatic signaling. The primary objective is to develop small molecules that can specifically and effectively inhibit assembly of this interface. Finally, the inventors are driven to pursue newer strategies that will not only target invasive cancers, but also help identify those with the worst prognosis before invasion is detectable. Identification of molecules that can serve both as therapeutic target(s) and biomarker(s) for prognostication will help tailor the therapy to the type of tumor, and thereby, pave a pathway to the practice personalized medicine.

The researchers have recently provided evidence that such central hubs do exist within signaling networks, from whence a novel class of multi-domain molecules could control the entire disease network, not just individual receptors. They christened these molecules as "rheostats". Four key properties define a molecular rheostat: Usually multi-domain in composition, they -- (a) directly bind to the cytoplasmic tails ofmultipleligand-activated receptors tointerceptincoming signals at the source of their origin, (b) activate trimeric G proteins in the vicinity of the receptor by virtue of its intrinsic GEF function tofine-tune(amplify/attenuate) signals via G protein intermediates, (c)transmitsignals within a major pathway by directly interacting with its key components (e.g., PI3K-Akt, MAPK-ERK, etc), andmost importantly, (d) aberrations in their expression that affect any of the above key functions have

been reported in key human diseases (e.g., oncogenesis, fibrogenesis, leukemogenesis). These properties empower rheostats to serve as highly significant targets in signaling cascades thatintercept, fine-tune, and transmitupstream signaling pathways,irrespective of the receptor of origin; well before the downstream cascade of dominos are set into motion. Mechanistically, it has been demonstrated that the interface between the G protein and one of these rheostats, via which the rheostat activates Gα-subunits, possesses a few fundamental traits that makes it an exciting therapeutic 'hot-spot' in invasive cancers, i.e., it is-- (a)specific, (b)sensitiveto disruption, (c)unique in the genome, (d)powerful and effective, because this interface isindispensablefor aberrant "amplification" of PI3K signals during cancer progression downstream of multiple oncogenic RTKs and GPCRs, (e)structural informationis available, and key residues in both Gαi and rheistat that participate in formation of the interface have been identified; and finally, (f) this rheostat is the first example of ametastasis-related genewhose interface with G➔i forms a promising candidate for the development of a targeted molecular therapy in the armamentarium against cancer metastasis. Partnered with computational modeling experts, some "drugable pockets" for the Gα-rheostat interface have been identified. A non-redundant database of 4,281,286 commercially available organic compounds were then screened against the obtained models using a standard ligand docking protocol. Using this method, some small molecules have been identified that could serve as potent antagonists of the Gαi-rheostat interface.
Cancer invasion/metastasis, diabetes, cardiac fibrosis, schizophrenia etc are all multigenetic diseases that is the end result of an aberrant signalling network, usually multi-receptor in etiology. To halt/ reverse these progressive diseases it will take more than just blocking one receptor/pathway at a time. Fundamentally, the development of silver-bullet therapies to treat these conditions needs identification and targeting ofmolecular hubsthat can modulate incoming aberrant signals from multiple receptors. We propose to target such a powerful hub, a unique protein-protein interface, which is dysregulated in each of these conditions, and primarily serves to modulate (amplify or attenuate) pathogenic signal networks triggered by multiple receptors. Targeting such hubs with agonists or antagonists will serve as tools for shifting the aberrant network in diseased cells back to a stable, physiological pattern. This is extremely important because most network-based therapies will help reshape the entire signaling network so that it lies in a new and stable region of behavior space.

Application area

UCSD researchers have developed and optimized an assay that follows the interaction between Gαi and an active fragment of rheostat (with its GEF motif). This assay could be re-formatted for high-throughput (HTP) mode (like an Alpha-screen). The adaptation of the GIV/Gαi interaction to the ALPHA screen will require the purification of GIV-CT protein or generation of GIV-GEF peptides, as well as purification of his-tagged Gαi3 (currently available). Once these peptides/proteins are purified they will be coupled to commercially available ALPHA beads and optimization of the assay for sensitivity and specificity will be performed. Once candidate small molecular inhibitors are identified, their efficacy can be evaluated in animal models of cancer invasion.

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