optimal timing for drug delivery. For all experiments involving LA, the group utilized non-neuroprotective doses of LA (0.005 mg/kg). For optimal drug concentration testing, Saleh et al synthesized 2 compounds, UPEI-200 and UPEI-201, composed of a 1:3 and 1:1 resveratrol:LA ratio, respectively. Following 6 hours of permanent ischemia, the animals were sacrificed and the infarct volume was determined using 2% 2,3,5-triphenyl tetrazolium chloride (TTC) staining.

In the first experiment, multiple doses of resveratrol (2 × 10−7 mg/kg up to 2 × 10−3 mg/kg) were administered 30 minutes prior to tMCAO. The authors also assessed infarct volumes when resveratrol was delivered 15 minutes following MCAO, as well as at 0, 30, 60, and 90 minutes following reperfusion. In all studies, comparisons were made to a vehicle control cohort. A statistically significant decrease of infarct volume based on the dose of resveratrol alone was seen at 2 × 10−4 mg/kg and 2 × 10−3 mg/kg (P ≤ .05), and the 2 × 10−3 mg/kg dose was effective even when delivered at 30, 60, and 90 minutes post MCAO (P ≤ .05).

The authors went on to demonstrate successful neuroprotection following the co-administration of resveratrol and LA (0.005 mg/kg) at various doses of resveratrol (2 × 10−7, 2 × 10−6, 2 × 10−5, 2 × 10−4, 2 × 10−3 mg/kg) in the same tMCAO model (Figure). Infarct volumes were also analyzed after the co-injection at delayed time intervals of 15, 30, 60, 90, and 120 minutes following MCAO. A dose-dependent reduction in infarct volume was observed when LA was co-administered with the 2 highest doses of resveratrol used (2 × 10−5 and 2 × 10−4, P ≤ .05). The authors then analyzed cerebral tissue sample for levels of cytoplasmic histone-associated-deoxyribonucleic acid fragmentation, a marker of apoptotic cell death. Compared to the control, LA combined with resveratrol showed a statistically significant decrease in cell death (P ≤ .05). Finally, UPEI-201 (resveratrol dose 2 × 10−6 mg/kg and LA dose 0.005 mg/kg) was shown to be effective when administered both 30 minutes prior to and at 15 minutes after MCAO in the tMCAO model.

The authors have convincingly demonstrated a dose-dependent reduction of infarct volumes by resveratrol administration following transient cerebral ischemia. When LA was administered in combination with resveratrol, the authors observed fewer necrotic cells in the core and diminished apoptotic neurons in the penumbra. The synergistic activity of LA and resveratrol produced neuroprotection in the tMCAO model at a 100-fold smaller dose of resveratrol and 1000 times smaller in the UPEI-201 trial. When these same synergistic doses were delivered individually, neuroprotection was not observed.

Such pharmacologic potentiation is being more frequently reported in the experimental stroke, cancer, and microbial literature. Qin et al analyzed hundreds of natural and synthetic anticancer and antimicrobial drugs to demonstrate synergistic effects at sub-therapeutic concentration. With the cost of healthcare at the forefront of most every political discussion, the discovery of an effective treatment to reduce stroke-associated disability is of paramount importance. Capitalizing on the synergism provided by co-administration of natural products as demonstrated by Saleh et al may ultimately provide an effective neuroprotective strategy.

Gregory M. Weiner, MD
Andrew F. Ducruet, MD
University of Pittsburgh Medical Center
Pittsburgh, Pennsylvania

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5. The Cyclin-Like Protein Spy1 Regulates Growth and Division Characteristics of the CD133+ Population in Human Glioma

The molecular characterization of adult brain tumors over the past decade has facilitated the development of targeted therapies for tumors with specific genetic fingerprints. For example, the identification of a methylated MGMT promoter gene in glioblastoma, a 1p/19q chromosomal co-deletion in anaplastic oligodendrogliomas, and mutations in the CDKN2A gene encoding the tumor suppressor p16, have each contributed to our understanding of brain tumor biology. In addition, such genetic profiles have enabled the development of targeted therapies specific for disrupting key steps in the growth patterns of primary brain tumors. Cyclin-dependent kinases (CDK) are a group of enzymes that play a key role in regulating patterns of cellular proliferation in normal cells. Disorders of CDK pathways have been demonstrated in many brain cancers. It is widely accepted that primary brain tumors are derived from a population of brain tumor-initiating cells (BTIC), which possess stem cell-like properties. Unsurprisingly, many of these BTIC have abnormal CDK proteins that permit dysregulated proliferation.

Recently, a highly conserved family of “cyclin-like” proteins, the Speedy/RINGO family, has emerged that is regulated developmentally and in a tissue-specific manner. These cyclin-binding proteins control the activity of the cyclin-dependent kinases, permitting orderly progression through the cell cycle. The originally characterized member of this family, known as Spy1, has been found to be required for cell cycle re-entry. Additionally, Spy1 has also been found to be dramatically upregulated in populations of proliferating astrocytes and microglia of the lumbar spinal cord. Interestingly, Spy1 levels are elevated in malignant human glioblastoma, and the levels of protein correlate with worsened prognosis. More recent research characterizes the role of Spy1 in the regulation of CDK activity in human glioma.

The investigators began by assessing the protein levels of Spy1 in oligoastrocytomas, oligodendrogliomas, and glioblastoma (GBM) vs control tissue from resected human samples. They found that Spy1 protein levels were progressively elevated with increasing tumor grade. Next they investigated the importance of Spy1 in brain tumorigenesis in vitro using U-87MG, SJ-GMBM2, and U251 glioma cell lines as well as samples obtained from 6 patients. Cells were infected with lentivirus carrying 2 different small hairpin RNA (shRNA) constructs against Spy1 (shSpy1) or a scrambled control (pLKO). Their results demonstrate that both of the Spy1-shRNA constructs reduced their targeted genes at least 3-fold and that Spy1-knockdown (Spy1-KD)
significantly reduced cell growth in all cell lines tested and in 4 of 5 primary lines.\(^6\)

The team then evaluated the role of Spy1 in terms of neurosphere formation capacity. They demonstrated that Spy1 expression levels were increased when cells were cultured in spheres with upregulation of the stemness markers Nestin and CD133\(^+\) as well as with down regulation of p27 and the oligodendrocyte marker NG2. Similarly, depleting U87 cell lines of Spy1 resulted in a marked reduction of CD133\(^+\) levels that occurred along with a decline in BTIC/proliferation markers GFAP, Ki67, c-Myc, and upregulation of p27 levels. Therefore, Spy1 appears essential for regulating the stemness properties of the CD133\(^+\) glioma population.\(^6\)

Given that Spy1-KD reduced expression of stem/progenitor markers and increased neuronal differentiation markers in several tumor models, the group then questioned whether Spy1 was endogenously regulated during differentiation. Harvesting neuronal stem cells from the sub-ventricular zone of post-natal mouse brains, the researchers grew cells in monolayers and neurospheres, then stimulated these colonies with growth factors. In both the monolayers and the neurospheres, Spy1 expression decreased with differentiation morphology and correlated with a decrease in stemness markers. Subsequently, Spy1 or an empty vector was overexpressed in these primary neuronal cells. They found that 60% of the control spheres had differentiated in 72 hours, whereas only approximately 20% of the Spy1 over-expressing neurospheres had differentiated (Figure). These results support that elevated levels of Spy1 favor expansion of stem/progenitor cells possessing glial characteristics and prevent functional differentiation toward a neuronal lineage.\(^6\)

Finally, the researchers assessed the effects of Spy1-KD on the self-renewal characteristics of BTICs in human glioma or tumor cell lines. Their results demonstrated that Spy1-KD caused a significant reduction in both the number and diameter of the resulting spheres. Additionally, this effect was reversible when a rescue construct for the Spy1-KD was used. Collectively, these data support the conclusion that endogenous levels of the Spy1 protein play a pivotal role in the promotion of neural stem cell self-renewal.\(^6\)

The ability to treat adult gliomas effectively is limited by our lack of understanding of the molecular pathophysiology of these tumors. However, this important research is an essential step forward that may facilitate our ability to create such targeted therapies. Preclinical and clinical trials will hopefully establish the feasibility of this approach in humans, and the research team should be heralded for their contributions, as their work provides a new avenue toward targeted tumor therapy.

Phillip V. Parry, MD
Johnathan A. Engh, MD
University of Pittsburgh
Pittsburgh, Pennsylvania

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