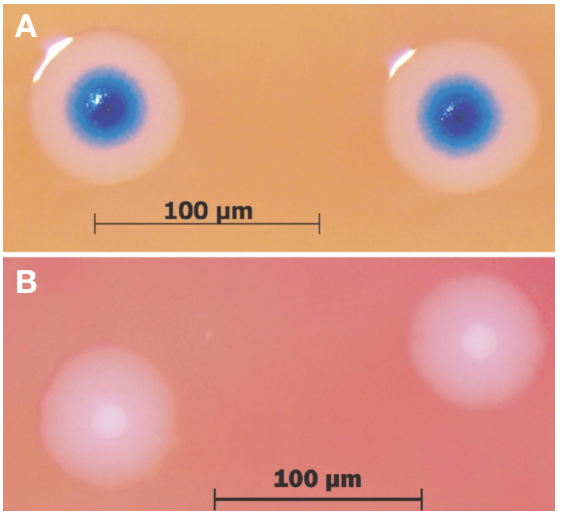
SCIENCE TIMES

Life, and . . . Neurosurgery After the First "Synthetic Cell"

Omne vivum ex ovo—meaning that all life arises from existing life, is a 17th-century dictum by the physician-physiologist William Harvey—has been regarded as a dogma until today. Nearly 400 years later, a group of 25 researchers from the J. Craig Venter Institute (JVCI), led by the renowned geneticist Craig Venter, call for a press conference to share a groundbreaking achievement: "*We're here today to announce the first synthetic cell,*" said Venter on Thursday, May 20, 2010, at the Newseum in Washington, DC. "*This is the first self-replicating cell we've had on the planet whose parent is a computer . . . We started with a digital code in a computer, built the chromosome from four bottles of chemicals, assembled that chromosome in yeast, and transplanted it into a recipient bacterial cell,*" Venter explained. Their work, a result of a 15-year effort, and approximately $40 million, was recently published in the journal *Science.*[1]



Three days after plating, the synthetic M. Mycoides *JCVI-syn1.0 colonies are blue because the cells contain the* lacZ *gene in their constructed genome and express beta-galactosidase, which converts the X-gal to a blue compound (***A***). The wild type* M. capricolum *cells do not contain* lacZ *and remain white (***B***).*
(Adapted from "Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome". Science 2010[1] *)*

Venter and his team, including the Nobel laureate Hamilton Smith, embarked on the quest with the ambition of building the "minimal" cell, containing only the minimal array of genes able to sustain life. Their journey was inaugurated in 1995, when they sequenced the genome of *Mycoplasma genitalium,* a bacterium with the smallest complement of genes of any known organism capable of independent growth.[2-4] Led by this initial goal, the team faced challenging technical hurdles, and made remarkable technological breakthroughs overcoming them, culminating in their current paramount discovery.[5-8]

To complete this final stage in the nearly 15-year process of constructing and booting-up a synthetic cell, JCVI scientists began with the accurate, digitized genome of the bacterium, *Mycoplasma mycoides*. The team designed 1078 specific cassettes of DNA, each of 1080 base pairs (bp). These DNA cassettes were designed so that the ends of each overlapped those of its neighbors by 80bp. They then employed a 3-stage process using their previously described yeast assembly system to build the genome using the 1078 cassettes.[5,6] The first stage involved taking 10 cassettes of DNA at a time to build 110, 10 000 bp segments. In the second stage, these 10 000 bp segments are taken 10 at a time to produce 11, 100 000 bp segments. In the final step, all 11, 100 kb segments were assembled into the complete synthetic genome in yeast cells and grown as a yeast artificial chromosome. The synthetic genome was largely identical to the native genome of *M. mycoides* with a few tweaks thrown in by the JCVI team. Those included "watermark" sequences and other designed gene deletions and polymorphisms, as well as mutations acquired during the building process.

The complete synthetic *M. mycoides* genome was isolated from the yeast cells and transplanted into *Mycoplasma capricolum* recipient cells, which were genetically engineered to lack their restriction enzymes. The initial synthesis and transplantation of the synthetic genome did not result in any viable cells. Following extensive, and complex troubleshooting, they were able to pinpoint a single point mutation in an essential gene responsible for the unsuccessful transplants. Once this single base pair error was corrected, the first viable synthetic cell was produced.[1]

The synthetic genome DNA was transcribed into messenger RNA, which in turn was translated into new proteins. The *M. capricolum* genome was either destroyed by the newly produced *M. mycoides* restriction enzymes or was lost during cell replication. Two days following the appearance of the first-self replicating cell, viable *M. mycoides* cells, containing only synthetic DNA, and proteins solely coded by it, were clearly visible on the petri dishes.[1,7,8] The transplanted synthetic DNA had rebooted the *M. capricolum* cells, converting them into a new *M. mycoides* strain. The researchers named the new strain *M. mycoides JCVI-syn1.0,* reminiscent of its computer-software ancestry. They called the new cells "synthetic" as, after about 20 divisions, not only the software (the constructed DNA they implanted), but also the hardware of those cells (all the proteins coded by the new DNA, as well as the other cell constituents produced by these proteins) was completely new.

Not only did Venter's audacious statements and claims of "synthetic" life mark a triumph of biotechnological ingenuity, but they also undermined the foundations of religions, cosmotheories, cultures, ethics, and law, questioning the essence of life itself. Naturally, these statements unleashed a storm of discussions in the scientific community and received enormous attention by the media. In fact, immediately after this announcement, the president of the United States, Barack Obama, asked the White House bio-ethics committee to report back to him in 6 months on the implications of this discovery.

Critics were quick to point out that the term "synthetic cell," Venter used, might have been imprecise. Many argued that although the new *M. mycoides* cells were uniquely genetically engineered, they were in fact descendants of the existing *M. capricolum cells;* as the cellular machinery of the latter was utilized transform them into the former.[9-11] Indeed, claims of creation of synthetic life can be found throughout history.[10] "*Frankly, scientists do not know enough about biology to create life,*" said James Collins, a biomedical engineer and a Howard Hughes Medical Institute investigator at Boston University in Massachusetts when interviewed by *Nature.*[9] "*We got the parts, we need a manual . . . It turns out that it is very hard to design even a two-gene network that performs in the way you would like it*" he added, explaining that the

major challenge lies in understanding the physiologic functions of the genes before getting many of them to work harmonically in a custom-designed genome.

The opinions of critics and researchers regarding the implications of the current report on our perception of life covered the entire spectrum. Some praised the event believing that it signifies the end of vitalism, whereas others downplayed the discovery to merely a technical achievement rather than a perceptual one. Between the two extremes, some concurred with the views of the researchers themselves, that the current breakthrough unprecedentedly increases our ability to explore the essence of life, as now we have the tools to design living organisms from scratch, limited essentially by our imagination[9].

The burgeoning field of synthetic biology, however, although unavoidably intermingled with cosmo-theoretical and life-definitional themes, has an ultimately utilitarian goal: to redesign the building blocks of life in order to serve the needs of humanity[11]. Venter's team already has a $600 million contract with Exxon Mobil to create a new strain of algae, capable of transforming CO_2 from the atmosphere and sunlight into fuel. In addition, they have funding from the National Institutes of Health to develop a future flu vaccine.

The technique, the JVCI team have devised, limits our ability of designing genomes to approximately 2 million bp, which excludes of course virtually any poly-cellular form of life. *"But two million base pairs is definitely within reach . . . and there are a lot of bacterial organisms out there that are useful and are within that range."* said Gibson, one of the main members of the JCVI team, when interviewed by *Nature.*[11] Contemporary genetically engineered organisms have no more than 10 of their genes altered. The researchers expect that the costs of designing and transplanting complete genomes will follow the same exponential decrease as DNA sequencing. Lower costs combined with automation will enable an explosion of synthetic genomics applications.[1]

The current breakthrough pins down most of synthetic biology's specific aims [12]; many of which having direct implications to medicine and to neurosurgery in particular. In the immediate future, a variety of biopharmaceuticals will be produced in bioengineered organisms, radically reducing the costs of production. Furthermore, designer bio-machines are already being tested as very sensitive and specific sensors for various kinds of infections. An example of one such sensor, are organisms that immediately change color when they detect MRSA in the CSF. In the near future, we expect upgraded techniques allowing for optimized control over the biofabrication of accurate 3D scaffolds, to which various kinds of cells attach (examples being chondrocytes in relation to artificial cartilage or peripheral nerves to their mesenchymal conduits). This has the potential to make engineered tissue of various kinds much easier to construct. Furthermore the realization of personalized drugs should not be that far away. Part of this development may well involve the fine-tuning of existing or new drugs to improve their therapeutic properties, and to produce low or no side effects for the individual. Further down the road, synthetic biologists hope to build biosensors, which permanently reside in the body to detect a particular type of abnormality; for example a type of cancer or arterial disease. The biosensor will be part of a genetically engineered machine, which then manufactures a drug to kill off the cancer cells or destroy the arterial plaque.[12] Many more applications are awaited in other fields such as agriculture, energy science, environmental safety and industry.[12]

A very worrisome topic surrounding the current discovery is the one of containment. Hazards arising from bio-error or bio-terror could prove devastating. The researchers expressed dread of the possibility that this technology could be used for terrorism. For the time being however, the construction and transplantation of whole genomes is extremely expensive, and remains beyond the technical capacity of most labs. In any case, multiple authorities are already working on legislations implementing the safe use of such technology.[9,11,13]

In conclusion, JCVI's work marks an unprecedented landmark in synthetic biology. Although criticized for the imprecise claims of synthetic life, their achievement takes the field of synthetic biology to another level. Their innovative technology is now arming scientists with powerful tools, enabling them to engineer innumerous practical applications for life, medicine and neurosurgery.

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Multiplicative Impact of Smoking and Genetic Predisposition on Intracranial Aneurysm Formation

In the era of preventive medicine, disease screening has both gained momentum and invoked controversy. Rational screening requires sophisticated knowledge in the areas of genetic predisposition and environmental risk factors as well as the interaction between these 2 factors. Knowledge in these areas vis-à-vis intracranial aneurysms (IAs) remains too primitive to justify routine screening. Nonetheless, recent genomewide association studies have shed light on specific single nucleotide polymorphisms (SNPs) associated with an increased risk of IA development. In addition, smoking has been indicted in multiple studies as the most important independent environmental factor associated with an increased incidence of IA. An interaction between smoking and genetic predisposition is plausible and would have implications for future screening protocols. With this in mind, Deka et al (***Stroke.* 2010;41(6):1132-1137**) sought to study the effect of smoking on the risk of developing an IA in a population with a known genetic predisposition.

White individuals with IA and a positive family history of IA were recruited from 26 international clinical sites. White control subjects known to be free of IA were randomly selected out of the greater Cincinnati population. Previously identified SNPs on chromosomes 2, 8, and 9 were genotyped. In total 6 variants were studied, 2 on each chromosome.

Four hundred and six patients and 392 control subjects were enrolled. Current smokers make up 47.3% of the patients while 35.2% were prior smokers vs 16.6% and 35.7%, respectively, in the control group. The strongest association was found with 1 of the SNPs on chromosome 8q. Both SNPs on chromosome 9p were found to be significantly associated with IA. The authors were not able to replicate the link between the variants on chromosome 2q and IA. Logistic regression analysis showed a multiplicative relationship between smoking and the high-risk alleles (Table).

TABLE. Logistic Regression Models on 3 SNPs and Smoking (Pack-Year) for Chromosomes 8 and 9[a]

SNP	OR	95% CI	*P* Value
Rs10958409 (chromosome 8q)			
Score per risk allele	1.48	1.06-2.07	.023
20 pack-years of smoking	5.04	3.50-7.61	<.001
Age per year	0.93	0.92-0.94	<.001
Rs10757278 (chromosome 9p)			
Score per risk allele	1.40	1.10-1.78	.007
20 pack-years of smoking	5.75	3.99-8.29	<.001
Age per year	0.93	0.92-0.94	<.001
Rs1333040 (chromosome 9p)			
Score per risk allele	1.37	1.08-1.74	.012
20 pack-years of smoking	5.16	1.88-2.71	<.001
Age per year	0.93	0.92-0.94	<.001

[a] SNP, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval. Models adjusted for age.

Although, the exact gene variants have yet to be determined, specific loci have been identified and confirmed in multiple studies and in populations with different ethnic and geographic backgrounds. What is also interesting is that the same regions on chromosome 9p are also linked with other arterial diseases such as myocardial infarction, coronary artery disease and abdominal aortic aneurysms highlighting potential commonalities between these different pathological processes. These commonalities could yield clues to a better understanding of the mechanisms behind IA formation.

We are still far from being able to develop rational screening protocols for intracranial aneurysms, but this study brings us a step closer. Certainly, cost benefit analyses and refinements in MRA sensitivity and specificity will be needed. Given the multifactorial nature of intracranial aneurysm disease, it is likely that a scoring mechanism based on environmental and genetic factors will be used in the future to guide screening, prevention and preemptive treatments.

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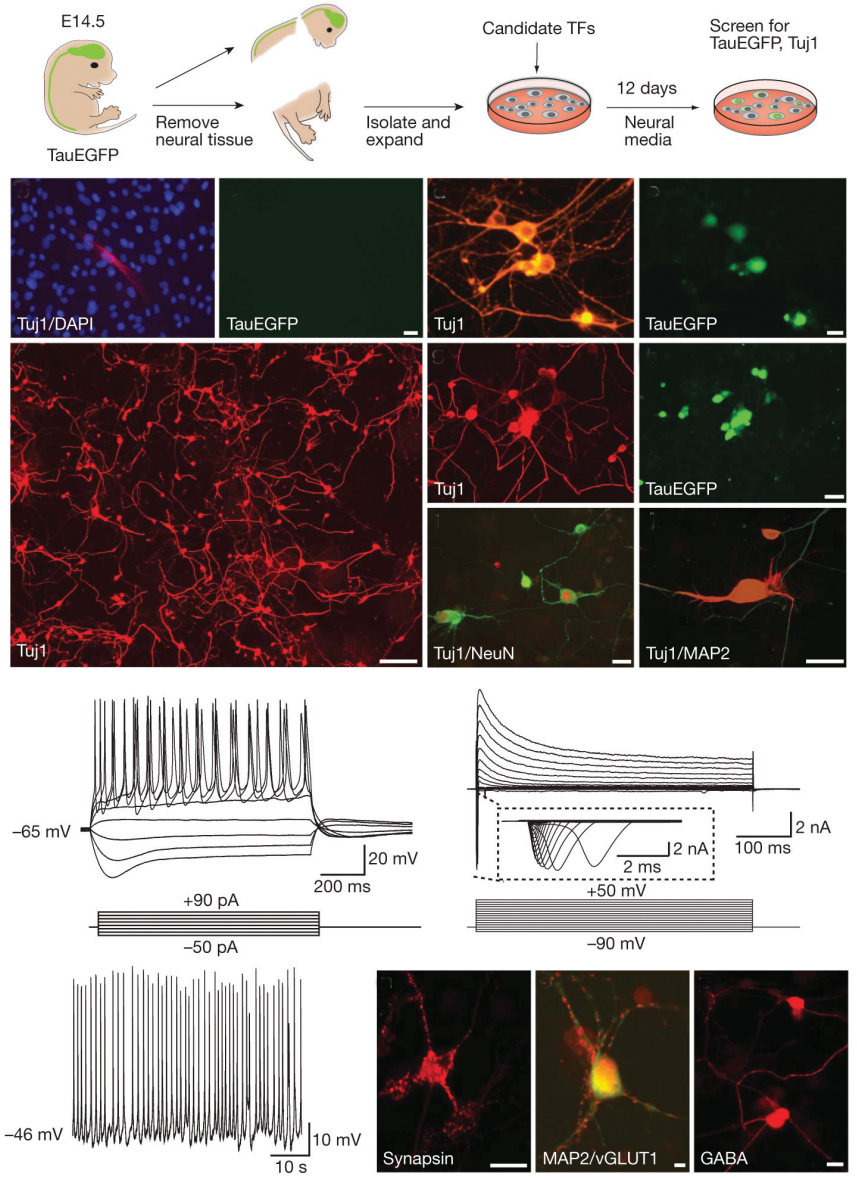
Turning Everything Into Brain?

The application of stem cell technology to create individualized therapies for patients promises to revolutionize medicine. Recent studies show that mature, differentiated cells can be reverted to a pluripotent state, thereby creating induced pluripotent stem cells (iPSCs). Such iPSCs can be differentiated into many tissue/cell lineages, and are useful both for modeling human diseases and possibly creating personalized cell-based therapies. A recent *Nature* paper reports another significant advance that bypasses the iPSC step, by demonstrating direct conversion of differentiated fibroblasts *directly into induced neuronal (iN cells)* **(Vierbuchen T, Ostereier A, Pang ZP, Kokubu Y, Südhif TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. 2010; 463(7284):1036-1041)**.[1] The major differences between these 2 techniques are highlighted in the Table. In the case of "pluripotent reprogramming," differentiated cells are induced through in vitro genetic manipulation to become pluripotent (able to form all cell types except germ cells) via de-differentiation. The regulatory genes required for reprogramming cells to iPSCs have been defined. Major limitations are that pluripotent cells cannot be induced in an in vivo environment, and carry significant risk of teratoma formation due to induction-related destabilization of the cellular epigenome and genome. In contrast, although iN cells are also formed though genetic manipulation of master regulatory genes in differentiated cells, they can be induced in vivo,[2] especially if the desired cell type naturally exists in the same tissue environment. iN cells are also hypothesized to be less teratogenic because these cells are created via trans-differentiation, and do not pass through an undifferentiated state like iPS cells. One notable challenge for the direct conversion process is that the genes necessary to direct such drastic cell type change are largely unknown, and will need to be identified for each differentiated cell type in future studies.

In the study published by Vierbuchen et al,[1] the authors identified a combination of optimal transcription factors, delivered via lentiviral vectors to mouse embryonic or tail-tip fibroblasts, that induced these cells to develop neuron-like morphology and behavior (Figure). Within 2 weeks post-infection with only 3 selected neuronal fate determination genes (Ascl1, Brn2, and Myt1l), the iN cells developed complex neurite morphology, generated both spontaneous and stimulus-induced action potentials, and exhibited expression of pan-neuronal markers and neurotransmitters such as glutamate and GABA. These and other tested transcription factor genes were previously shown to be important for neural development. Additionally, iN cells were able to form functional synapses with a preexisting cortical neuron network and with other iN cells when cultured with primary astrocytes. Characterizing changes in action potential height, neurite morphology, resting membrane potential, and other measurements over time demonstrated that iN cell development is highly analogous to normal cortical neuronal development. Overall, this is the first study that shows conversion of differentiated fibroblasts directly into cells with mature neuronal biological properties. Similar experiments have induced muscle specific properties in fibroblasts.[3] Some caveats to consider are that this pioneering study was done in vitro and it remains to be seen if similar results can be attained in vivo. One possible method might be to develop optimized viral targeting of cell-specific surface markers to provide induction specificity in generating the desired cell type(s). Further, it will be important to ascertain whether such direct cell fate conversion remains stable over the long term without additional manipulations. The use of lentiviral vectors carries the risk that some cells incorporate multiple copies of the regulatory genes into their genome which may result in late genetic instability. However, newer methods have been developed to bypass genome manipulation to produce iPS cells through direct application of transcription factor proteins[4] or through episomal vectors.[5] Perhaps these methods could be adapted to create iN cells. Finally, this study shows results obtained in mice, therefore the viability of this technology with human cells remains to be proven.

The clinical implications for this work span the fields of regenerative medicine and neurosurgery. Foremost is the possibility of creating an individualized, renewable source of lineage-specific cells for studying disease, testing therapies and possibly for cell-based treatments. It is difficult and impractical to obtain and culture differentiated neuronal cell types from patients, especially after trauma, stroke or degenerative disease processes. iN cells may be useful in creating regenerative therapies such as creating iN cells for repair after strokes, or creating motor



*Characterization of mouse embryonic fibroblast (MEF)-derived induced neuronal (iN) cells. (**A**) Experimental set-up. The authors used a Tau-EGFP (enhanced green fluorescent protein) knock-in mouse model in which all neuronal cells express EGFP at the tau gene locus. MEFs were isolated (EGFP-negative) and cultured. Cloned transcription factor genes were delivered to cells via lenti-viral vectors. Days post-infection, genes producing MEFs that are now EGFP–positive were assessed for neuronal features. (**F-J**) 12 days post-infection with optimal 5 gene pool (Ascl1, Brn2, Mytl1, Olig2, and Zic1)–iN cells show complex neurite morphology and express pan-neuronal markers (Tuj1, β-tubulin; NeuN, neuronal nuclear protein; MAP2, microtubule-associated protein). (**K**) iN cells show action potentials through step current depolarization. (**L**) iN cells show evidence of fast inactivating sodium currents (inset) and outward potassium currents. (**M**) iN cells produce spontaneous action potentials that can be blocked by tetrodotoxin. (**N-P**) iN cells produce excitatory (glutamate) and inhibitory (GABA) neurotransmitters as well as express synapse specific protein synapsin.*

neurons to treat amyotrophic lateral sclerosis or spinal cord injury. The distinct advantage is that such cells are patient-derived and would avoid immune rejection. This work demonstrates a new paradigm that may be useful in creating novel biological, individualized therapies, especially for repairing and restoring the function of the nervous system.

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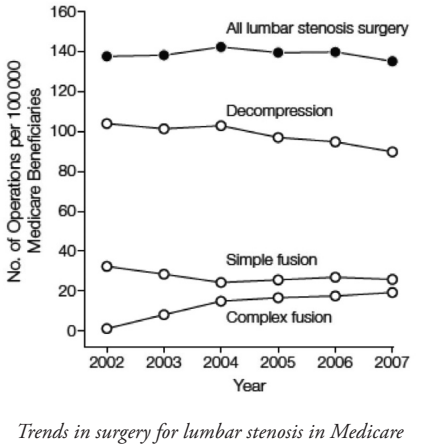
Surgery for Spinal Stenosis in Older Patients: What's the problem?

Spinal stenosis is a leading cause of pain and disability in older populations and is a growing problem. Treatment involves physical therapy, analgesics and anti-inflammatories, injection therapy, and surgery. Recent class I evidence from a randomized controlled trial demonstrated a distinct benefit in outcome from surgery compared to non-surgical therapy in patients with lumbar stenosis, but various surgical techniques are available and wide variations seem to exist in which technique is used and when. With a renewed interest in maximizing clinical efficacy with reduced cost and complication, investigators based at the Oregon Health and Science University led by Deyo reported their findings on trends, complications and charges associated with surgery for lumbar stenosis in the April 7 issue of JAMA (**JAMA. 2010 Apr 7;303(13):1259-65.**).



Trends in surgery for lumbar stenosis in Medicare patients from 2002-2007. Simple decompression and fusions declined while complex fusion rose.

Deyo et al performed a retrospective cohort database analysis using Medicare claims data from 2002-2007. A total of 32 152 patients over the age of 65 were grouped into 3 surgical categories: decompression alone, simple fusion (1 or 2 levels, single approach), or complex fusion (more than 2 levels, or combined anterior/posterior approach). Covariates included age, sex, race, previous hospitalization, morbidity score, chronic pulmonary disease, previous spine surgery, number of disc levels treated, scoliosis, and spondylolithesis. Outcomes included readmission, wound infection, stroke, heart attack, mortality, length of stay, and discharge to nursing home status.

In this report, though the overall number of simple decompressions and simple fusions dropped, the number of complex fusions increased almost 15-fold. Increased complications and mortality were associated more complex surgeries, but also with previous spine surgery, chronic pulmonary disease, higher comorbidity score, and with hospitalization in the previous year. Factors associated with having had more complex surgery included scoliosis, spondylolithesis, and multiple disc levels, but increased complications rates were still seen when considering only patients with stenosis alone who received complex procedures. Overall, medical complications rose from 2.3% to 5.6% and rehospitalization from 7.8% to 13% in patients with simple decompression vs complex fusion, respectively. Adjusted odds ratio for life-threatening complications for complex fusion vs simple decompression was 2.95 (confidence interval, 2.5-3.49).

These findings led the authors to conclude that surgeons are opting for more complex operations and that this is due in part to "financial incentives to hospitals and surgeons for more complex procedures."

Unfortunately, clinical outcome measures evaluating improvements in pain, function and quality of life are lacking in this analysis, thus it is impossible to say whether or not the trends observed have lead to improved patient outcomes or not. The only class I data on surgery for lumbar stenosis showed a benefit with surgery (including simple decompression with or without fusion) versus no surgery even after 2 years.[1] In addition, there remain deficiencies in the data used for their anaylsis. The financial calculations in their study were based upon hospital charges, not cost. Charges carry a variable relationship to actual costs of care delivery and other factors may have affected this parameter over the 2002-2007 study period.

Overall, the results of this report indicate that the rate of complex fusions for lumbar stenosis in Medicare patients is rising and that there is a corresponding rise in medical and surgical complications with more complex surgeries. The reason behind this shift in performing more complex surgeries is unclear. Determining the proper selection criteria for which surgery to perform in which patients is obviously important to maximize patient safety and avoid unnecessary costs, and should be a priority for spine surgeons. It is important to remember that surgery for lumbar stenosis is both effective and beneficial. The trick is to balance surgical ability with informed judgment.

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Classification of Genomic Changes in Breast Cancer Brain Metastasis

Most breast cancer patients will die from their metastatic disease. In fact, as new drugs have succeeded in reducing systemic disease (HER-2 targeting monoclonal antibody-Trastuzumab), the brain has increasingly become the site of untreatable relapse. Although the majority of brain tumors are metastases, with primary gliomas representing a small minority, there is a notable dearth in biological understanding and investigation of how metastatic cells colonize the brain.

Recently, The Cancer Genome Atlas group has successfully categorized human GBMs into the following subtypes: classical, neural, proneural and mesenchymal. Employing a similar investigational approach and sequencing technologies, Mardis and et al **(Ding et al *Nature* 2010)** have characterized and compared the genomic changes in a biopsy sample from the primary tumor of a patient with basal-like breast cancer and a sample from a cerebellar metastasis that developed 8 months later. They looked for structural variations, alterations in the copy number of genomic regions and differences in the distribution of mutations among the samples *[Figure 4 from paper]*.

From these observations, several conclusions were made: (1) most of the original genomic changes present in the primary tumor are propagated during the clinical course of the disease, (2) the different mutation frequency observed in the tumors is an indication of genetic heterogeneity in the cellular population of the primary tumor, (3) and two different populations of cells from the primary tumor that had a distinct subset of mutations that were selected for in the metastatic processes.

In the cascade of events underlying metastasis to the brain, successful colonization of the inhospitable brain habitat is the most challenging component. Although, vascular dissemination of tumor cells occurs readily, a variable period of dormancy ensues prior to relapse in the brain. This clinical scenario suggests that most disseminated tumor cells are not imme-

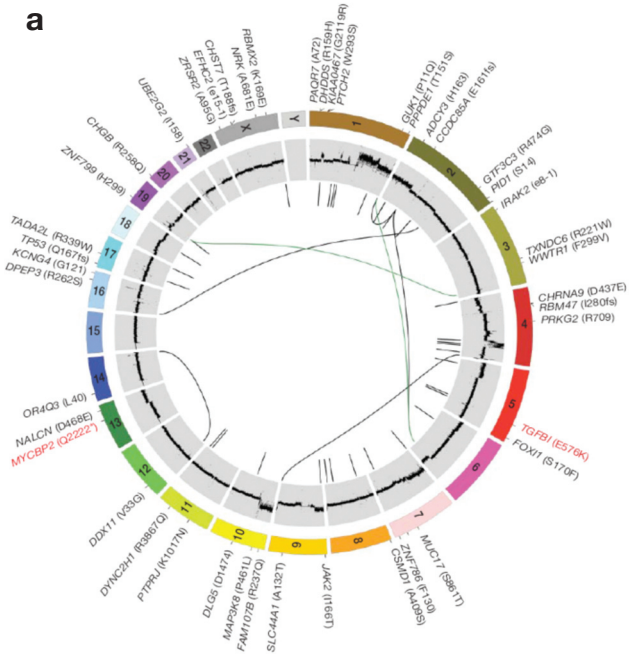

Figure 4. Circos plots for the primary tumor, metastasis and xenograft genomes. **a-c**, *Circos[30] plots display the validated tier 1 somatic mutations, DNA copy number and validated structural rearrangements in the primary tumor (***a***), metastasis (***b***), and xenograft (***c***). Mutations enriched in the metastasis or xenograft are in red in panels* **b** *and* **c**. *Mutations and the large deletion unique to the metastasis are in blue (***b***). Translocations only present in primary tumor and metastasis are in green. All shared events are in black. The copy number difference between the tumor and normal is shown (scale; –4 to 4). No purity-based copy number corrections were used for plotting.*

diately competent for generating macrometastses; and any effort to understand the underlying mechanisms must include the brain with its unique blood brain barrier and microenvironment. As such, insights from neuroscientists and neurosurgeons will help provide the soil from which innovative and interdisciplinary investigative approaches arise.

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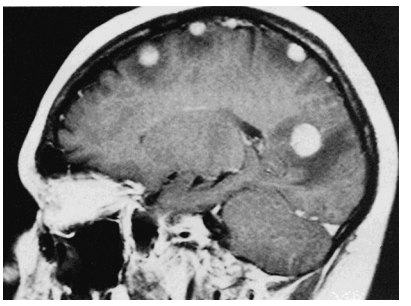
Optimal Radiotherapy in Patients With Multiple Intracranial Metastases

The best treatment option for patients with multiple intracranial metastatic lesions remains to be determined. Previous studies have shown the benefits of both surgery, whole brain radiation (WBRT), and stereotactic radiosurgery (SRS).[1-5] In a review of 502 patients treated with WBRT-RS or WBRT, Sangahvi et al. demonstrated that SRS significantly improve survival in patients with intracranial metastasis.[6] Although SRS appears to be beneficial, the necessity of WBRT is uncertain. In order to answer this question, a previous study randomized patients with brain metastasis to receive WBRT plus SRS (65 patients) or SRS alone (67 patients). The 12-month brain tumor recurrence rate was 46.8% in the WBRT-SRS group and 76.4%for SRS alone group (P = .001). Salvage brain treatment was less frequently required in the WBRT-SRS group (n = 10) than with SRS alone (n = 29, P = .001). Despite these differences there was no significant difference in median survival time or the 1-year actuarial survival rate between groups (7.5 months and 38.5% in the WBRT-SRS group and 8.0 months and 28.4% for SRS alone).[7] This again questions the necessity of WBRT in patients with metastatic brain tumors.

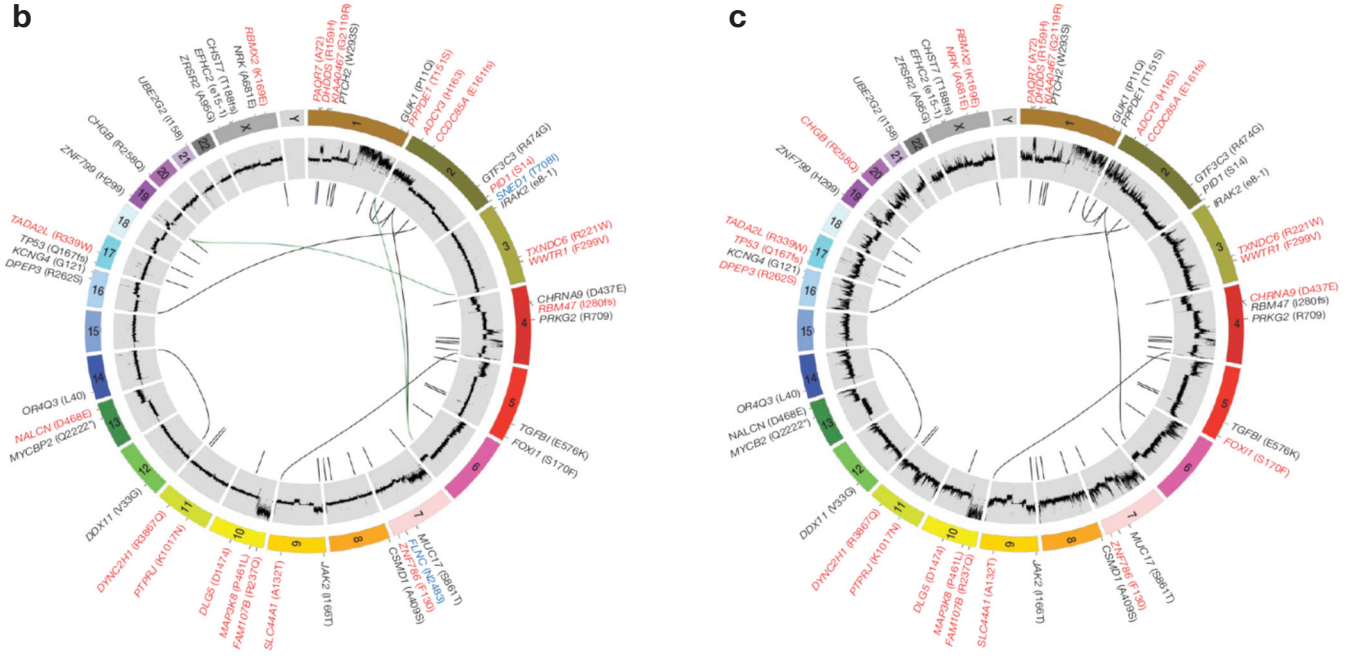
To investigate the best therapy for patients with multiple brain metastases, the Japan Leksell Gamma Knife (JLGK) Society has planned a prospective multi-institute study (Japan Leksell Gamma Knife JLGK0901) for selected patients with 1 to 10 brain lesions. The plan for this trial is based on a recently published retrospective analysis.[8]

Among 1,918 patients with metastatic brain tumors treated by GKS from January 1998 through May 2009, 778 satisfied the JLGK0901 study inclusion criteria: (1) newly diagnosed brain metastases; (2) 1–10 brain lesions; (3) less than 10 cm^3 volume of the largest tumor; (4) less than 15 cm^3 total tumor volume; (5) no magnetic resonance (MR) findings of cerebrospinal fluid (CSF) dissemination; and (6) no impaired activity of daily living (<70 Karnofsky Performance Score [KPS]) due to extracranial disease. At initial treatment, all lesions were irradiated with SRS without upfront WBRT. Thereafter, enhanced MR imaging (MRI) was applied every 2 to 3



months, and new distant lesions were appropriately retreated with SRS or WBRT. Patients were divided according to tumor number: single lesion for group A (280 cases), 2 for group B (135), 3 to 4 for group C (148), 5 to 6 for group D (93), and 7 to 10 for group E (122). The primary organ was lung in 579 patients, gastrointestinal tract in 79, breast in 48, urinary tract in 34, and others/unknown in 38.

On multivariate analysis, significant poor prognostic factors for overall survival (OS) were active systemic disease, poor (<70) initial KPS, and male gender. Neurological survival and qualitative survival at 1 year were 92.7% and 88.2%, respectively (7). Incidence of new distant lesions at 6 months and 1 year were 69.8% and 43.8%, respectively. There were statistically significant differences in new lesion emergence between groups A and B and between groups B and C. SRS using GK provided good results in selected patients with 1



to 10 brain lesions, without prophylactic WBRT. Tumor control rates at 1 year were 98.4% in tiny, 92.3% in small, and 77.9% in medium-sized tumors ($P < .0001$). Mean survival time was 0.83 years in lung cancer, 0.65 years in gastrointestinal (GI) tract, 0.91 years in breast, 0.68 years in urinary tract, and 0.41 in other/unknown origin (NS). There was also no significant difference in mean survival time according to number of metastases: 0.83 years for 1, 0.69 years for 2, 0.69 years for 3 to 4, 0.59 years for 5 to 6, and 0.62 years for 7 to 10 metastases.

In the proposed prospective trial twelve hundred cases will be prospectively registered from 23 Japanese GK sites within 2 years and a 1-year follow-up period will be mandatory. The protocol stipulates follow-up, including enhanced MRI and neurological examinations, at least every 3 months. This study is designed to prove the noninferiority of overall survival with several metastases in groups D and E (5 to 10) vs 3 to 4 metastases in group C. Based on power analysis, a planned total sample size of 1200 has been proposed for this trial.

The retrospective study by Serizawa et al. revealed that the number of brain lesions has no effect on survival.[8] Although these results are anticipated to be confirmed by the final results of the JLGK0901 study, further analysis is warranted. There may be differences in cohort broken down further by size or type of tumor, which may effect treatment plans and outcomes. This trial may also provide further information about the optimal treatment for specific subgroups further subdivided by size or type of lesion. Further studies will be necessary to determine the optimal treatment, if any, for patients with larger tumors, imaging evidence of cerebrospinal fluid (CSF) dissemination, and impaired activities of daily living.

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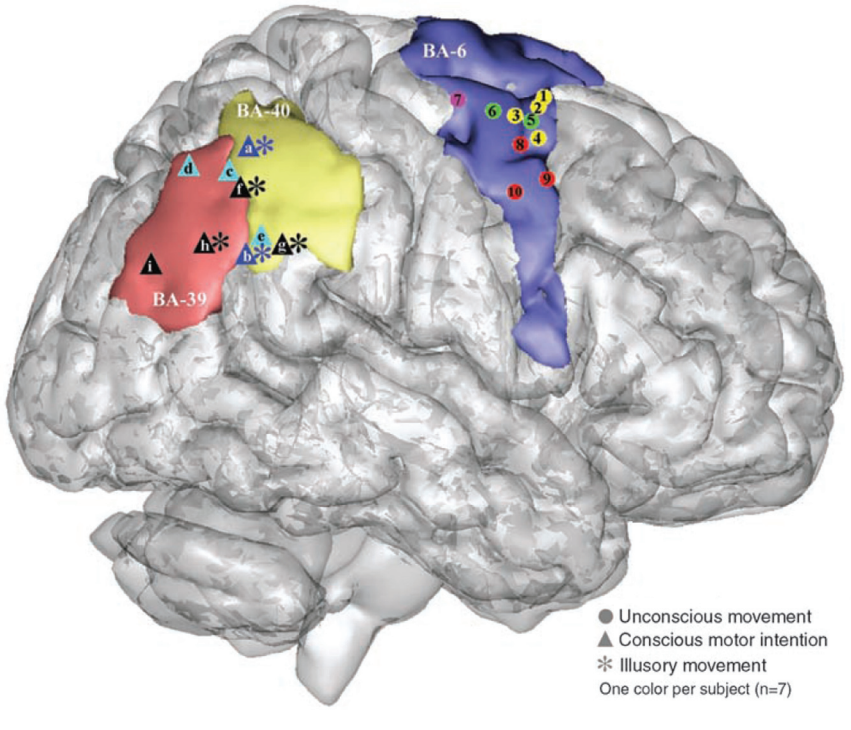
Exciting Neurons: Controversies in Cortical Stimulation

In 2009, Desmurget et al published an interesting study (**Desmurget M, Reilly KT, Richard N, Szathmari A, Mottolese C, Sirigu A. Movement intention after parietal cortex stimulation in humans. *Science*. 2009; 324(5928):811-813.**) attempting to characterize the origin of human movement intention, and via cortical stimulation in patients undergoing awake craniotomies, these authors demonstrated strong subjective feelings of movement intention by stimulation of the inferior parietal cortex (Brodmann's 39 and 40). At higher stimulation currents, the subjects even reported the completion of these movements when in fact no electromyographic activity was observed (Figure). The substrate of movement intention could also lie in premotor cortical regions, however direct stimulation here in this study never resulted in subjective feelings of intention, only overt movements at high stimulation intensities with frequent denial of the movements by the subjects. Recently, these findings have been criticized however in an interesting exchange of opinions.

In the 2009 paper, Desmurget et al describe the patients undergoing parietal stimulation experiencing strong subjective feelings of the intention of movement with each subject using phrases such as "will," "desire," and "wanting to" when asked to characterize their sensations. This result seems to align with primate studies showing parietal involvement in motor planning tasks. The authors further discuss that the hypothesized forward modeling function of the posterior parietal regions might also play a role in the subject's insistence that movement had taken place during higher stimulation currents. Recently however, in a criticism of this article, Karnathet al have pointed out that direct cortical stimulation might not simply cause cortical activation as interpreted by Desmurgetet al and in fact several other studies. Deactivation is another possibility, and this could result in removing disinhibition from another remote structure responsible for movement intention.

The specific criticisms of Karnath et al (***Science*. 2010 Mar 5;327(5970):1200; author reply**



Stimulation induced responses in both the tested parietal and premotor regions. Left-sided stimulations are shown on the right hemisphere. BA-40 is yellow, BA-39 is orange, and BA-6 is in blue.

1200) on the interpretation of cortical stimulation experiments revolve around the probability that an observed biophysical effect likely represents a complex sum of excitatory and inhibitory influences caused by the stimulation. These competing effects are due to the precise geometry and field gradient values at the location of axon initial segments are passing fibers, and to support this tenet the authors outline various published positive and negative phenomena produced by cortical stimulation. In an accompanying technical comment, Sirigu et al (***Science*. 2009 May 8;324(5928):811-813.**) counter that their experimental results might be explained by distant or inhibitory effects, but the wealth of data from other sources (including neuroimaging, neuropsychological findings, and lesioning studies) support the direct action of the posterior parietal cortex in movement intention and awareness.

Sirigu et al go on to point out that it is unlikely that remote recruitment of premotor cortex could be responsible for the subjective feelings of movement produced during parietal stimulation, since direct stimulation of the premotor cortex does not reproduce the effects, and stimulation of the posterior parietal regions does not produce any spinal cord activation which you would expect from disinhibiting primary motor cortex. Additionally, as opposed to language production or visual object processing (which can be disrupted by stimulating connected regions), there was no ongoing behavioral task for the set of subjects studied during parietal stimulation. The subjective feelings occurred de novo and represent an evoked positive behavioral response.

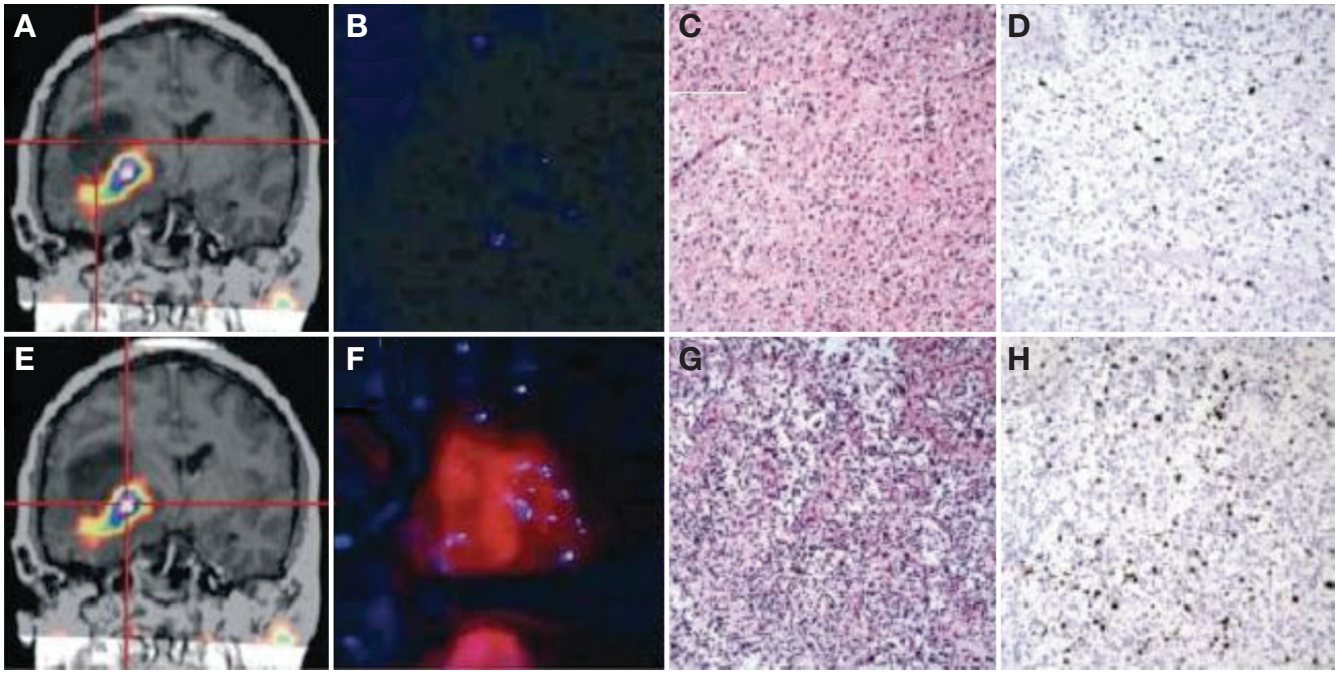
In summary, this conversation between Sirigu et al and Karnath et al points out how often difficult it is to interpret human cortical electrophysiology experiments based on existing clinical stimulation techniques. It will be critical for neurosurgeons to derive an understanding of how direct stimulation affects the active awake cortex, both for its future therapeutic uses as well as diagnostic uses in the operating room.

William Anderson

Improving Intraoperative Visualization of Anaplastic Foci Within Gliomas

Following surgical resection or stereotactic biopsy of a glioma, adjuvant chemotherapy or radiation is initiated following assessment of the histological diagnosis and grading according to the World Health Organization (WHO) criteria. The decision to implement chemotherapy, radiation therapy, and/or experimental interventions is largely based upon the final pathological diagnosis. In many cases, tumoral heterogeneity can contribute to inaccurate tumor grading, which can subsequently hinder initiation of appropriate adjuvant therapy. For example, a grade 3 astrocytoma can potentially be diagnosed as a grade 2 astrocytoma due to sampling error, thus delaying the appropriate administration of fractionated irradiation.

5-aminolevulinic acid (5-ALA) is a prodrug which induces the accumulation of fluorescent protoporphyrin IX in malignant glioma cells[1]. The agent is available in Europe, and is pending clearance by the food and drug administration for generalized application in the United States. Interestingly, 5-ALA does not generally accumulate in non-malignant gliomas (i.e. WHO 2 fibrillary astrocytomas). The intraoperative augmentation of tumor visualization with 5-ALA has been shown to improve the degree of resection of malignant gliomas in a randomized trial[2]. In a more recent study performed at the Medical University of Vienna (***Cancer* 2010; 116(6):1545-52**), Widhalm et al. assessed the



Illustrative case of a woman with an insular glioma. Panel **A** *demonstrates the coronal view from intraoperative navigation into an area of the tumor with no enhancement and no enhanced metabolism on positron emission tomography (PET). Panel* **B** *shows the intraoperative microscopic view, with no fluorescence under violet-blue excitation light. Histopathology from this area (***C***) is consistent with a World Health Organization (WHO) 2 astrocytoma on hematoxylin and eosin-stained slides, and (***D***) MIB-1 labeling index is 5%. In contrast, panel* **E** *demonstrates an area of high metabolism on PET (still non-enhancing on MRI), from the intraoperative image guidance display. This area demonstrates obvious fluorescence (***F***) under the operating microscope. Pathologic specimens from this particular area (***G*** and ***H***) demonstrate an anaplastic area (***G***) with an MIB-1 of 14.5% (***H***). Modified, from Widhalm G, Wolfsberger S, Minchev G, et al. 5-aminolevulinic acid is a promising marker of detection of anaplastic foci in diffusely infiltrating gliomas with nonsignificant contrast enhancement.* Cancer *2010;116(6):1545-1552.*

ability of 5-ALA administration to differentiate anaplastic foci within gliomas with no evidence of focal pre-operative contrast enhancement[3]. Such tumors are often difficult to stage appropriately because of intratumoral heterogeneity.

5-ALA is generally administered as an oral solution prior to general anesthesia induction for tumor resection. During tumor resection, a modified operating microscope is required to visualize tumoral fluorescence. This microscope has the ability to convert from standard white light visualization to violet-blue light, which delineates the fluorescent cells. In a total of 17 cases of WHO 2 and WHO 3 gliomas, none of which demonstrated focal contrast enhancement on pre-operative magnetic resonance imaging (MRI) scans, the authors discovered that focal 5-ALA fluorescence was a meaningful factor differentiating grade 2 and grade 3 tumors. None of the grade 2 tumors demonstrated any evidence of fluorescence. 8 of 9 grade 3 tumors demonstrated focal fluorescence, surrounded by 5-ALA negative areas. The MIB-1 index was significantly higher in 5-ALA positive areas of the tumor (Figure).

This study provides a possible opportunity to more effectively identify anaplastic gliomas among brain tumors in the absence of focal enhancement. As long as histopathologic grading remains the gold standard for the determination of appropriate treatment for these patients, the use of biomarkers such as 5-ALA may help to more accurately grade these tumors and allocate appropriate therapies for them. As our molecular understanding of these tumors continues to evolve, it will be of interest to determine what the relationship is between tumoral fluorescence and molecular profiling data.

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Embolus Extravasation: A New Mechanism for Microvascular Recanalization?

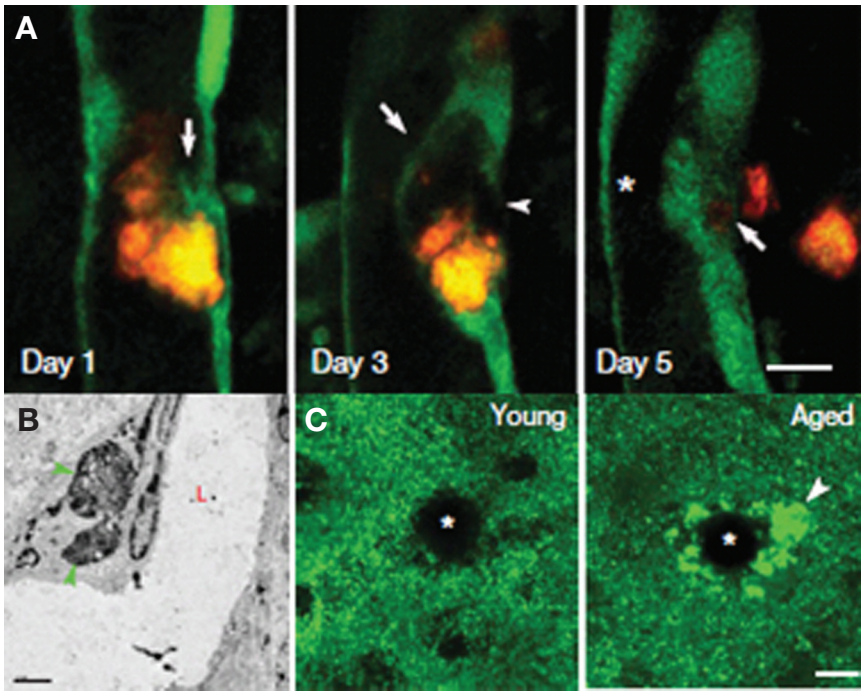

Figure **1A**. *A fluorescently-conjugated microembolus was first enveloped by an endothelial membrane by Day 3, then extravasated from the microvessel by Day 5 (Adapted from Figure 2A of Lam et al).*
Figure **1B**. *Transmission electron microscopy showed extravasated fibrin clot (arrow heads) and patent vessel lumen (Adapted from Figure 1J of Lam et al).*
Figure **1C**. *Confocal images of synaptophysin immunoreactivity near an occluded microvessel (asterisk) showed dystrophic synapses (arrowhead) in aged mice (Adapted from Figure 4C of Lam et al).*

Patency of cerebral vasculature is essential to normal brain functions. Occlusion of large intracranial arteries can lead to devastating consequences of cerebral ischemia and infarction, whereas small vessel occlusion caused by microemboli was found to be frequently associated with dementia and cognitive decline.[1,2] A number of clinical studies with Transcranial Doppler Ultrasonography have demonstrated that silent embolic events in the brain are common especially in patients with carotid or aortic arch atherosclerotic disease.[3,4] Throughout evolution, the body has developed effective counter-acting mechanisms, such as the fibrinolytic system, to degrade abnormally formed blood clots so as to ensure uninterrupted blood flow. However, these innate pathways seem less robust in terminal arterioles and capillaries due to smaller size of the microvessels and low velocity of blood flow in the capillary bed.[5-7] Are there alternative means to efficiently re-canalize the microvasculature in the brain? This question was answered by a most recent study published in *Nature,*[8] in which a group of scientists from Northwestern University School of Medicine described a direct extravasation pathway that clears small emboli from cerebral microvessels.

The authors used mice expressing endothelial-specific green fluorescent protein (Tie2-GFP) as subjects and developed a system of two-photon microscopy and electron microscopy to image the microvessels. They first observed that, after fluorescently-conjugated microemboli (8-20 μm) were infused through the carotid artery of a mouse, a substantial number (50-60%) were cleared within 2 hours, presumably by the fibrinolytic system and hemodynamic forces. However, the retained emboli were difficult to be washed off in the subsequent days and usually caused cessation of blood flow in the occluded microvessels. After extending the in vivo imaging interval, the authors found that, by day 6, many emboli were no longer located inside the vessel lumen and the blood flow had resumed. This process of extravasation seemed to start as early as 2 days after embolization and could last for 2 to 5 days (Figure, A). It frequently led to complete re-establishment of blood flow and sparing of the vessel (70-80%). These observations from *in vivo* imaging were confirmed by transmission electron microscopy where microemboli were found to have translocated outside of the vessel lumen (Figure, B).

Next, the authors investigated the underlying mechanism by which embolic material was extravasated from the microvasculature. They observed that within 24 to 48 hours, the embolus was completely enveloped by an endothelial membrane projected from the adjacent endothelial wall. Then, the original endothe-

lium underwent retraction via tight-junction disruption to allow the passage of the enclosed embolus (Figure, A). The newly-formed endothelial membrane covering the embolus made frequent contact and transient adhesion with the opposing endothelium, and eventually a new vessel lumen was established and blood flow resumed. Moreover, it was also demonstrated that matrix metalloproteinases (MMPs) were important mediators of the extravasation process, probably because of their proteolytic functions in the degradation of tight-junction and extracellular matrix, and that inhibition of these enzymes markedly decreased the rate of embolus extravasation.

Furthermore, the authors reported that age was a significant factor on the efficiency of embolus removal and the severity of neuronal and vascular damage surrounding the occluded microvessel. While younger mice (4-month-old) were able to clear most microemboli and suffered no obvious vascular degeneration or neuronal injury, older mice (22-month-old) showed much less robust extravasating capability. Dystrophic synapses (Figure, C) and Caspase-3 immunoreactive peri-vascular cells were also frequently observed in older mice after embolus injection, suggesting that persistent microemboli could eventually lead to permanent capillary and neuronal loss in the brain.

The delayed extravasation described in this paper is a new and independent mechanism by which occluded cerebral microvessels can be recanalized via translocation of the microemboli. It is especially useful to clear occluding materials not susceptible to fibrinolysis such as cholesterol or atherosclerotic fragments. It may also play an important role in neurodegenerative diseases and cerebral vasospasm.[1,9,10] While the underlying genetic and molecular pathways are still unclear, the findings of this study provide additional insights into the microenvironment surrounding the cerebral vasculature in thromboembolic events.

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