Advances in the Diagnosis and Treatment of Malignant Brain Tumors

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Malignant brain tumors are frequently lethal despite aggressive therapeutic approaches, including surgery, radiation therapy, and chemotherapy. Over the years only marginal impact on the natural history of brain tumors was achieved, with survival measuring in months to a few years for the most common tumor types. Nevertheless, new diagnostic, surgical, and other treatment modalities are being constantly developed in an attempt to improve survival or provide a cure for this disease.

The diagnosis of brain tumors has been revolutionized with the advent of modern imaging modalities, such as computerized tomography and magnetic resonance imaging scans. Nonetheless, new challenges and demands are being presented to diagnostic radiologists. Better resolution of anatomical details of the brain is required. Clinicians seek functional imaging that will enable detection of the degree of metabolic activity and blood flow within tumors, localization of functional or eloquent brain regions, and translation of radiological data for real-time, intraoperative use by the surgeon. Radiation oncologists demand better radiation planning to minimize side effects and enhance efficacy of radiation, while medical oncologists explore new effective drugs against brain tumors with alternative delivery techniques to bypass the limitations posed by the blood-brain barrier.

Advances in Diagnosis

The rapidly developing computer technology and mathematical modeling have enabled the creation of powerful diagnostic tools. MRI scanners use stronger magnetic fields, advanced software applications, and more powerful computing capabilities that are able to provide precise anatomical details nearly identical to photographic details of corresponding anatomical sections of the brain. The software used in modern machines also enables dynamic studies, such as obtaining cine mode images that show actual cerebrospinal fluid flow in the subarachnoid space. However, the most impressive new application using MRI technology is functional imaging.

Functional MRI

Functional MRI takes advantage of minute changes in blood flow and levels of oxygenation that occur in activated brain regions [1]. These changes can be depicted by MRI to delineate precisely the anatomical correlate for specific functions. For example, a patient is instructed to perform various motor tasks with his right hand while being scanned. The processed scan will detect the exact site of his contralateral motor cortex. The same approach can be taken for detecting speech centers, as well as for other important functions. These data are invaluable for the surgeon who treats patients with tumors that reside within or adjacent to these eloquent brain regions. By knowing the locations of these sites beforehand, the surgical plan can be modified in a way that will minimize the risk to the patient.

Nuclear medicine

Other modalities of imaging can provide important data regarding the metabolic activity of brain lesions depicted by CT or MRI scans [2]. Nuclear medicine specialists can use radioisotopes to indicate the metabolic status of tumors and thus differentiate between tumoral and non-tumoral conditions (such as brain lesions that result from radiation damage, degenerative or ischemic lesions, and other non-neoplastic conditions). PET (positron emission tomography) and SPECT (single photon emission CT) are diagnostic techniques that are currently in use for these purposes [Figure 1A].

Images obtained from any of the above mentioned modalities can be fused with conventional CT or MRI data into one set of images to provide an integrated anatomical, functional, and metabolic image of the brain.

Surgical Advances

The complex three-dimensional nature of the brain has been a major hurdle for neurosurgeons who perform aggressive surgical resections of brain tumors that reside in deep or eloquent regions. Accordingly, many tumors were deemed inoperable and surgical resections were often incomplete due to the fear of causing a devastating neurological deficit to the patient. In this setting, the introduction of computer technology and intraoperative imaging as a neurosurgical enhancing tool has been stupendous, transforming science fiction into science fact. These technologies of image-guided surgery are giving surgeons

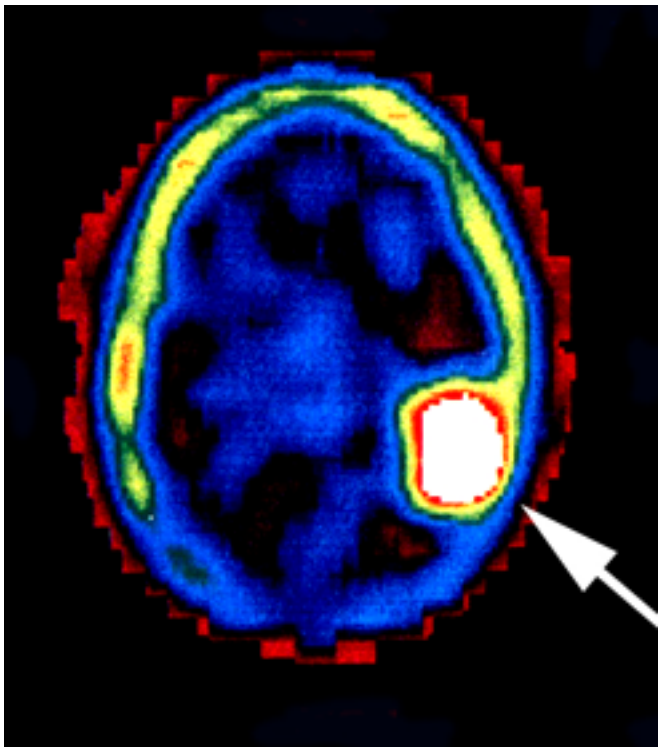

Figure 1A. A thallium SPECT of a patient with a left parietal weakly enhancing lesion. The scan demonstrates intense uptake of Thallium–201, indicating a hypermetabolic lesion (arrow) proven during operation to be an anaplastic astrocytoma.

X-ray vision, allowing them to remove tumors more effectively while enhancing the safety of the procedures and avoiding damage to critical tissues.

Neurosurgical navigation

Considering the fact that operations on the brain are carried out through small openings of the cranium and that the position of the head differs from one operation to another, the mental reconstruction that was traditionally used by neurosurgeons to identify their location within the brain was far from satisfactory. Neurosurgical navigation is a term used to describe the ability of the surgeon to know at any given time the coordinates of his operating tools in the three-dimensional volume of the brain.

Several systems of surgical navigation using different technologies have been developed in recent years. Basically, these systems use a probe held by the surgeon that can be visualized by two or more cameras at a known distance from one another and which are located at some distance from the operating field. The cameras capture signals emitted from the probe, and a computer system linked to the cameras can calculate the exact position of the probe in the brain and display it on an anatomical model. This anatomical model (the brain) is provided by feeding the computer with imaging data of the patient (CT or MRI) obtained just prior to the operation [Figure 1B]. Modifications of the software developed for these procedures are being used by oncologists to plan and execute

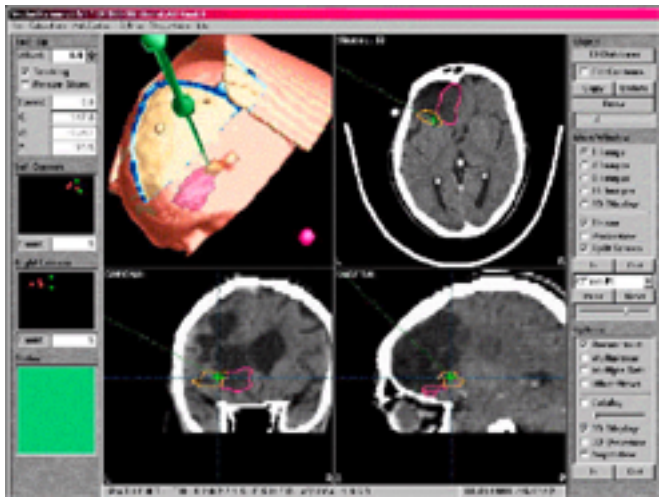

Figure 1B. The control screen of the computerized surgical navigation system (BrainLAB Vectorvision®, Germany) during an operation for resection of a left hemispheric recurrent astrocytoma. The motor speech area was predetermined by functional MRI and is illustrated in yellow. The tumor mass is depicted in red. The green mark illustrates the real-time trajectory and position of the surgical instrument used by the surgeon.

complex radiation plans using conventional radiation or radiosurgical techniques [3]

Intraoperative MRI

One major drawback of surgical navigation is that the anatomical model used by the computer is based on images obtained before the operation. Once the surgeon removes part of the brain or causes movement of the brain by aspiration of cerebrospinal fluid for example, the accuracy of the navigation is greatly reduced. Accordingly, other means to provide up-to-date, real-time images of the brain during surgery are necessary.

Intraoperative MRI is one such means [4]. Because the operation takes place in the MRI, new scans of the patient can be obtained on a continuous basis to allow for frequent checks of the surgeon's progress. Thus, the surgeon may determine with accuracy when complete removal of the tumor has been achieved, when eloquent brain regions are encroached upon, and — by inherent navigation capabilities — can determine his or her exact location on a continuous basis.

An initial prototype of an intraoperative MRI has been developed by *General Electric* and is being used in about 10 medical centers worldwide, including the Sheba Medical Center in Israel [Figure 2]. The MR machine is built such that the magnet is split in two, allowing a space for the patient's head and the surgeon to be located between them. This technology required the substitution of all equipment used during surgery (tools, operating microscope, anesthesia apparatus, etc.) with equipment made of non-magnetic materials. The operating room itself needs to be shielded from radiofrequency disturbances. About 50 neurosurgical operations have been performed so far in the open MRI at the Sheba Medical Center, the majority

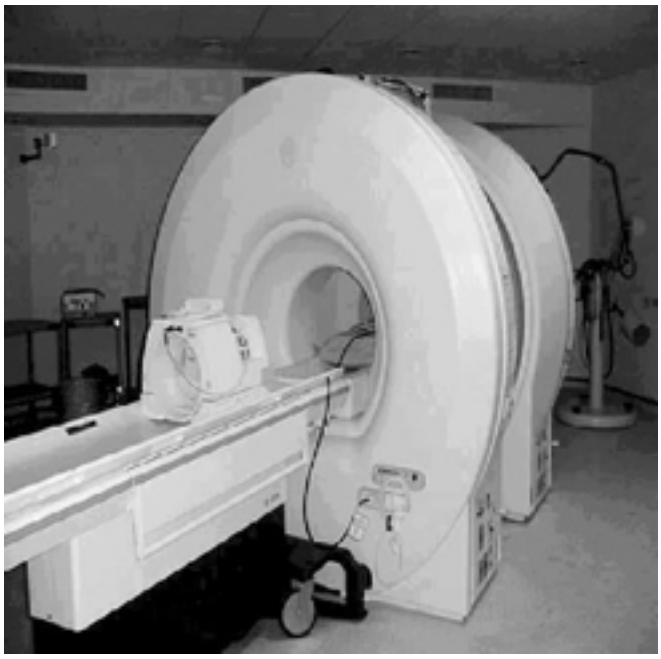

Figure 2. The *General Electric* open iMRI (interventional MRI) at the Sheba Medical Center. All equipment seen in this operating room is specially made of magnet-free materials.

of them for resection of complicated brain tumors [Figure 3].

In an attempt to simplify the use of intraoperative MRI, new machines designed to be incorporated into regular operating rooms are currently being designed and tested. One such "portable" intraoperative MRI, manufactured by the Israeli-based Odin Technologies company, uses a weak magnetic field (0.12 Tesla) and allows introduction and removal of the MRI into the operating field on demand, while providing high resolution images of the surgical field [Figure 4].

Intraoperative brain mapping and awake craniotomy

While not a completely novel procedure, intraoperative brain mapping in awake patients was an arcane technique for many surgeons until its recent popularity and application in increasing numbers of neurosurgical centers worldwide. This technique entails surgery using local anesthetics only to enable communication with the patient during the entire operative procedure. This is especially important when an operation is performed within, or adjacent to eloquent brain regions, such as areas involved in language or motor functions. After exposing the brain, the cortex is stimulated with an electrode to detect the exact location of the functional area. These areas are then protected, and the incision sites are selected in non-eloquent regions in order to reach a deeper tumor. Thus, tumor resection can be performed without damaging critical brain regions, thereby preventing postoperative neurological deficits. Recovery from such operations is usually rapid, with many patients discharged within 24–48 hours

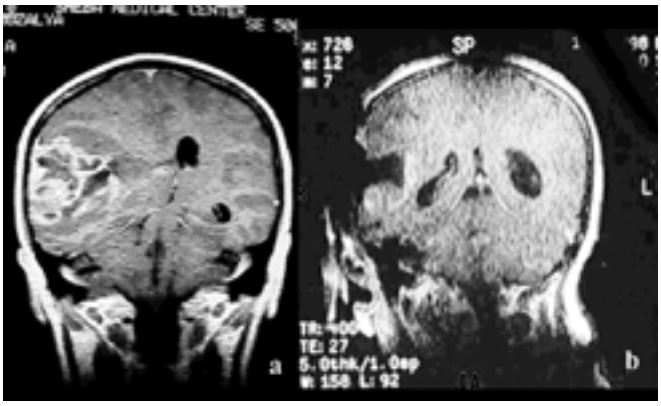

Figure 3. Real-time MRI images generated during surgical resection of an extensive right hemispheric anaplastic oligodendroglioma in the open iMRI (interventional MRI). [a] Preoperative coronal MRI scan after injection of gadolinium, showing the large enhancement.

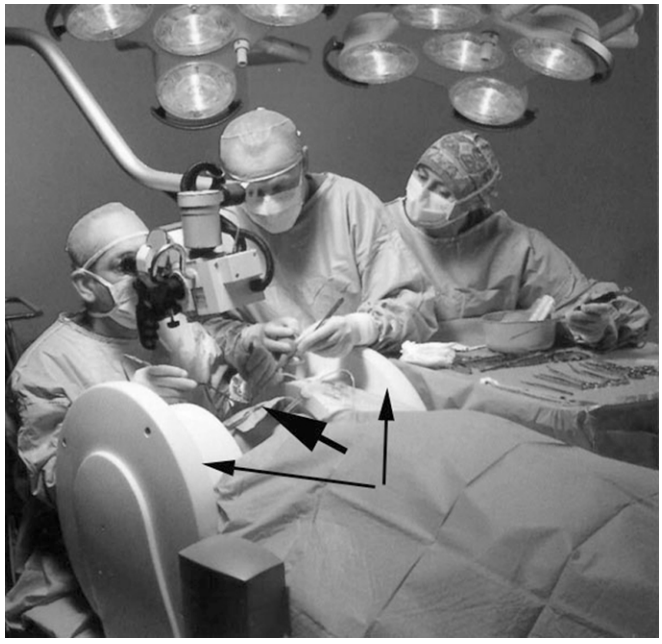

Figure 4. A small-scale intraoperative MRI scanner developed by Odin Technologies (Yokneam, Israel) that can be used on demand in a regular operating suite. The long arrows indicate the separated discs of the magnet. The short arrow indicates the position of the patient's head during the operation

after surgery. Combining awake craniotomy and mapping with neurosurgical navigation may even facilitate and enhance the success rate of these operations.

Intracranial endoscopy

Endoscopy has gained popularity in many fields of general surgery and has recently been introduced to neurosurgery as a standard technique for selected applications [5]. This *minimally invasive* technique can be used in some patients whose brain tumors are within or adjacent to the ventricular system, for complete resection of the tumor, diagnostic biopsies, or as an adjunctive tool to conventional microsurgical resection of tumor (endoscopy-assisted surgery) [Figure 5]. Since the entry port for insertion of the endo-

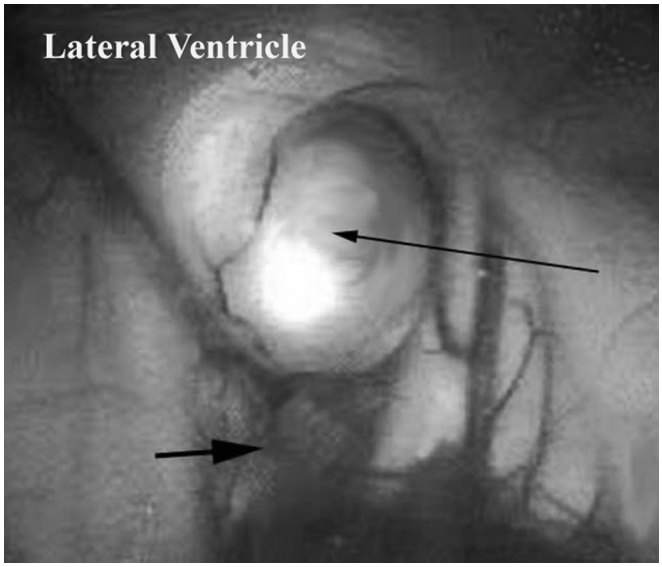

Figure 5. An endoscopic view of the lateral ventricle. The long arrow illustrates a large colloid cyst within the foramen of Monro that can be completely removed using the endoscope. The short arrow depicts the choroid plexus compressed by the tumor.

scope is small (about 1 cm), the morbidity associated with this type of surgery is very low and recovery is usually swift.

Experimental Therapies

A review of experimental therapies currently under investigation for the treatment of brain tumors is beyond the scope of this review. However, some of the major approaches that are being explored will be briefly discussed.

Delivery of chemotherapy

Malignant gliomas are known to be refractory to systemically delivered chemotherapeutic drugs. One of the main reasons for this lack of response is the poor penetration of drugs across the blood-brain barrier.

Several strategies have been attempted to improve the penetration of chemotherapeutic drugs into brain tumors. These include intra-arterial and intrathecal administration, CSF perfusion, the use of biodegradable chemotherapeutic polymers, direct intratumoral application, blood-brain barrier disruption, convection-enhanced delivery of drugs, and liposomal packaging. Some of the more novel approaches are described below.

- ***Biodegradable polymers.*** This material, which is mixed with a chemotherapeutic agent and then implanted within the cavity after resecting the tumor, undergoes degradation, causing sustained drug release that is proportional to the polymer erosion rate. Clinical trials using polymers containing nitrosurea-based chemotherapy (GLIADEL®, Rhone-Poulenc-Rorer, France) for recurrent malignant glioma have demonstrated a modest survival advantage in patients treated with the polymers as compared to patients treated with placebo polymers [6]. A large-scale multicenter study using Gliadel wafers in newly diagnosed glioma patients has recently been concluded, the results pending within the next few months. The benefits of this method include circumventing the blood-brain barrier, minimal systemic toxicity, and elevated drug concentrations within the tumor mass itself. However, the limited distance of diffusion of the drug may limit the efficacy of this approach.
- ***Blood-brain barrier disruption.*** The concept of BBBD[1] has been developed over recent decades and is based on transient opening of the barrier to allow enhanced penetration of chemotherapeutic drugs into the CNS, allowing the tumor to be exposed to a higher concentration of the drug for a longer period [7,8]. Up to 100-fold increase of drug delivery can be achieved with intra-arterial delivery after BBBD compared to systemic, intravenous administration of the drug. Mannitol has been the most commonly used mode of disruption, which acts by causing transient osmotic shrinkage of endothelial cells, causing the tight junctions to separate. Second-messenger systems such as calcium influx are also affected by the changes brought about by this osmotic shrinkage. Its use is not without risk and has been associated with cerebral ischemia and infarction, elevated intracranial pressure, and acute brain edema. While its efficacy has been clearly demonstrated in several brain tumor types, such as lymphoma and germ cell tumors, its value in the therapy of malignant gliomas is still controversial. Other, non-hyperosmolar compounds have been evaluated for their capacity to disrupt the blood-brain barrier. Bradykynin and several bradykinin agonists can specifically activate receptors on endothelial cells. Clinical studies using the bradykinin B2 agonist RMP-7 in patients with glioma are currently in progress [9,10].
- ***Enhanced-convection drug delivery.*** A promising new technique for distribution of solutions into brain tissue is fluid convection, or bulk flow, which occurs in the brain interstitial fluid under normal conditions, with vasogenic edema, and after infusion of solutions directly into brain parenchyma [11]. It has recently been shown that fluid convection within the brain, established by maintaining a pressure gradient during interstitial infusion, can be used to supplement diffusion and greatly enhance the distribution of various molecules, including high molecular weight proteins. These studies suggest that using convection to supplement simple diffusion enhances distribution of large and small molecules in the brain, while achieving *in situ* drug concentrations that are orders of magnitude greater than those attainable by systemic administration. Convection can be used to supplement diffusion for distribution of certain compounds to treat much larger volumes of brain than can be achieved by

[1] BBBD = blood-brain barrier disruption

diffusion alone, and with great pharmokinetic advantage over systemic administration. This technique for drug delivery may also enhance distribution of various anti-cancer drugs in tumors of the CNS. Several clinical studies using this technique are currently being evaluated.

Gene Therapy

Recent advances in understanding the molecular basis of diseases along with technical innovations in recombinant DNA technology have paved the way to the newly evolving era of gene therapy. In its simplest definition, gene therapy is the process by which genetic material is transferred into somatic cells to bring about a therapeutic effect. This can be achieved by either replacing defective or missing genes or by introducing new functions to the host's cells.

Many disorders of the central nervous system may serve as potential targets for gene transfer therapeutic approaches. These include tumors, metabolic diseases (especially hereditary inborn errors of metabolism), degenerative disorders such as Parkinson's disease, and various inflammatory and infectious diseases.

The realization that multiple genetic alterations occur during the process of tumor formation, including CNS tumors, has directed attention to the use of gene transfer as a therapeutic option. Most gene therapy approaches attempting to treat brain tumors have used viruses for *in vivo* gene transfer to tumor cells. A variety of viral vectors have been used in pre-clinical studies, but retroviral vectors and adenoviral vectors are currently the main vector systems used in clinical trials.

Five major gene therapy approaches for therapy of brain tumors are currently being explored. These include gene transfer-mediated drug targeting (suicide gene therapy), transfer of tumor suppressor genes and cell cycle modulators, genetic immune modulation, anti-angiogenic gene therapy, and the use of cytopathic/oncolytic viruses.

Suicide gene therapy has been the most commonly employed technique in clinical trials for treating brain tumors. It involves the conferring of drug sensitivity by transducing tumor cells with a gene encoding an enzyme that can metabolize a non-toxic prodrug to its toxic form.

HSVtk, the herpes simplex virus thymidine kinase gene, converts non-toxic nucleoside analogs, such as ganciclovir, into phosphorylated compounds that are used to build the DNA molecule. These compounds directly inhibit DNA polymerase and render the formed DNA molecule unstable, leading to arrest of DNA synthesis and cell death. Thus, glioma cells genetically modified to express will be killed after administration of ganciclovir. Preclinical experiments have demonstrated that marked tumor elimination occurred despite gene transfer into only a limited fraction of the tumor cells. This cytotoxic effect of transduced cells on adjacent non-transduced cells was termed the "bystander effect." This "bystander effect" is mediated mainly by transfer of toxic phosphorylated forms of ganciclovir to non-transduced cells, presumably via gap junctions. Another presumed mechanism contributing to the bystander effect includes targeting of mitotically active endothelial cells in tumor vessels by the retroviral vector and subsequent zones of tumor infarction after their destruction with ganciclovir administration. An immune-associated anti-tumor effect was also suggested when this approach was applied to experimental animals. Clinical trials using this approach [12] have demonstrated a modest anti-tumor effect in relatively small tumors, with no significant toxicity. However, the main problem of *in vivo* gene transfer remains the inability to efficiently deliver genetic particles into solid tumors. Alternative modes of delivery, such as intravascular, may be needed to achieve successful therapeutic gene transfer.

Other ongoing gene therapy clinical studies are evaluating the role of transferring genes involved in cell-cycle modulation and growth control to induce apoptosis or enhance the response of modified tumor cells to radiation therapy [13]. *TP*53 is one such gene.

Summary

Malignant brain tumors are tough to treat. The plethora of novel technological advances and the multitude of experimental therapies are indicative of this reality. However, it becomes clear that no magical panacea will provide the cure, and only step-wise progression of research and improved technological advances to enhance the efficacy of surgical and adjuvant therapies will eventually improve the outcome of brain tumor patients.

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Capsule

Ligand binding

How does the binding of a molecule to a cell-surface receptor change the biochemistry inside the cell? To test exactly how the receptor protein moves when the ligand binds, Ottemann et al. from Berkeley strategically attached spin labels to various parts of the aspartate receptor. The relative movement of these probes after aspartate binding revealed that one of the transmembrane helices of the receptor moved in a piston-like fashion about 1 angstrom relative to the other transmembrane helix to influence intracellular events. This tiny motion appears to rule out other likely mechanisms, such as rotation, association, or a scissor or seesaw movement of the receptors.

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Capsule

Ribozymes in the nucleolus

Ribozymes are RNA molecules that behave as enzymes, severing other RNAs at specific sites into smaller pieces. They may be valuable therapeutic tools for repairing cellular RNAs transcribed from mutated genes or for destroying unwanted viral RNA transcripts in the cell. However, targeting ribozymes to the cellular compartment containing their target RNAs has proved a challenge. Samarsky et al. report that a family of small RNAs in the nucleolus (snoRNAs) can readily transport ribozymes into this subcellular organelle.

There are two major classes of snoRNA, each with its own highly conserved sequence motif. The C/D box snoRNAs regulate 2'-O-methylation of the ribose sugars of ribosomal RNAs (rRNAs), and the H/ACA box snoRNAs guide pseudouridylation of rRNA uridine bases. A few snoRNAs also participate in processing precursor rRNA transcripts. Most snoRNAs are transcribed and processed in the nucleus, although some may be synthesized in the nucleolus (the nuclear site of rRNA synthesis).

Samarsky et al. chose yeast for their experiments because the requirements for trafficking of a specific snoRNA (called U3) are well understood in this organism. They showed that nucleolar localization of the yeast U3 snoRNA was primarily dependent on the presence of the C/D box motif. The investigators appended a test ribozyme to the 5' end of U3, and then inserted its RNA target sequence into the same location in a separate U3 construct. So, both the ribozyme and its target were expressed in separate, modified U3 snoRNAs. The snoRNA-ribozyne molecule (called a snorbozyme) and its U3-tethered target were transported into the nucleolus. Here the ribozyme cleaved its target RNA with almost 100% efficiency.

SnoRNA chimeras harboring ribozymes or protein-binding elements should prove valuable not only therapeutically but also for elucidating why certain RNAs and proteins traffic through the nucleolus.

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