## Session I | Now and future in oral cavity cancer surgery

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| **Precision surgery: New definition for future surgical oncology** | 정한신 (성균관대) |
| **Image-guided surgery** | 김석원 (성균관대 외과) |
| **Cancer surgery using NGS information** | 이동진 (한림대) |
| **Surgery combined with Immunotherapy** | 정우진 (분당서울대) |
| **Reconstruction through tissue engineering** | 권성근 (서울대) |

# Precision surgery for cancer: A new surgical concept in individual tumor biology-based image-guided surgery*

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## INTRODUCTION

Surgery has long been a cornerstone of cancer treatment in many types of cancer. The main goal of surgical cancer treatment is to completely remove the tumor without remnant cancer cells. Achievement of tumor-free margins is essential to reduce the risk of local recurrence; meanwhile positive resection margins (the presence of cancer cells at the resection margin) is one of the most significant negative predictors for recurrence and survival in various cancers.[1-4] Furthermore, poor treatment outcomes related to positive resection margins may not improve after salvage surgery or adjuvant treatment.[1-5]

In this manuscript, we reviewed the use of optical tools during surgery with regard to the goals of surgical resection for cancer treatment and sought to define a new surgical concept: precision cancer surgery as an extension of image-guided surgery.

### 1. Limitations of current surgical treatments

Traditionally, intraoperative assessment of the resection margin is largely dependent on visual inspection and palpation of tumors, with the aid of frozen section analysis. Although preoperative imaging such as computed tomography (CT), ultrasonography (US) and magnetic resonance imaging (MRI) can provide gross anatomical information, in situ translation of these images to the operation field is challenging. Even with intraoperative frozen section analysis, overall diagnostic accuracy is not high due to sampling error and limited amount of samples and examination.[6,7] Due to these factors, the incidence of close (less than 5-mm safety margin around tumors) and positive margins are as high as 40% in head and neck cancers.[8]

### 2. Image guidance for precise cancer surgery

With the advancement of molecular imaging technology and its clinical application, the gap between preoperative radiologic images and surgical findings has been reduced through image- guided surgery.[9-11] The primary purpose of image-guided surgery is to visually differentiate tumor cells from the surrounding tissues in real time, which enables complete resection and preservation of normal function to the greatest extent possible.[12]

*This article was submitted to the Precision and Future Medicine, a SKKU MED journal.

### 1) Conventional imaging-guided surgery

Intraoperative delineation of tumors using conventional anatomical imaging modalities has been applied to tumor ablative surgery for several solid cancers. Glioblastoma is a highly infiltrative brain tumor with poorly defined tumor boundaries. Preoperative MRI is limited to defining tumor margin clearly during surgery because of the brain shift and swelling that occurs during surgery.[13] To overcome these shortcomings of preoperative imaging, intraoperative MRI has been introduced into the neurosurgical field. Many studies have reported that intraoperative MRI-guided surgery improves resection quality without increasing neurological complications and has a positive impact on survival.[13-15] In a randomized controlled trial, intraoperative MRI offered 96% total resection in comparison with 68% without it.[13] However, the need for multiple resources for intraoperative MRI may be a stumbling block to clinical application in other types of cancers.

Breast conservation surgery has been recognized as a standard surgery for early breast cancer.

Similar to other solid cancers, positive resection margins are a significant poor prognostic factor for recurrence and survival.[16-18] A randomized clinical trial comparing intraoperative US- guided surgery to palpation-guided surgery revealed significant improvement in securing negative resection margins with ultrasonography during surgery (3% vs. 17% positive resection margins favoring ultrasonography-guided surgery).[18] In addition, US-guided surgery reduced the resection volume with good margin status, suggesting that it enabled more accurate surgery.

In short, intraoperative radiology or ultrasonography-guided surgery has the potential to improve the primary goal of surgery using anatomical information during surgery. However, it has some limitations such as the low anatomical resolution of conventional imaging for surgery, inability to capture anatomical displacement or distortion during surgical manipulation, difficulty of real-time imaging during surgery (except intraoperative ultrasonography), and a lack of cancer-specific information.

### 2) Fluorescence-guided surgery

Fluorescence optical imaging with various imaging probes and molecular targeting materials has been introduced for real-time image-guided surgery to overcome the drawbacks of conventional imaging. These technological developments enable the surgeon to remove the cancer at a submillimeter level.[12,19] The characteristics of cancer cells including increased transformed optical properties, growth factors, angiogenesis, and proteolysis have been utilized in fluorescence optical image-guided surgery.[20] This allows real-time feedback during surgery even in the surgical wound bed. As a result, it can provide wide surgical field images, and can detect microscopic residual disease.

#### (1) Conventional fluorescence-guided surgery

In a fluorescence-guided surgery, tumor-specific signals are the main target for visualization to discriminate between tumor and normal tissues.[21] Thus, signal to background ratio (or tumor to background ratio) is a key component of optical imaging. Molecules with fluorescent light emission are known as fluorophores, whereas molecules with light absorption are called chromophores.[20] The representative chromophore in cancer imaging is hemoglobin, which absorbs light spectra less than 600 nm in wavelength. Water and lipids absorb light spectra over 900 nm.[20] Fluorescent imaging using the visible light spectrum (400- to 600-nm wavelength) has high nonspecific background light with scattering and a low tumor to background ratio,[22] which frequently fails to meet the im-

aging criteria (tumor to background ratio >3).[23]

(2) Near-infrared fluorescence imaging

A light spectrum between 650 and 900 nm is more desirable for fluorescence imaging, because less absorption in normal tissue and a relatively high tumor to background ratio.[24] In addition, this near-infrared light spectrum has high tissue penetration (5-10 mm) with little interference from intrinsic fluorescence.[10] Thus, nonspecific fluorescence can be minimized and tumors can be delineated more clearly during surgery. In line with the improvement in near-infrared fluorescent imaging, several imaging systems have been introduced in the clinical setting to visualize near-infrared signals.[25-27]

## 3. Fluorescent probe for image-guided surgery

Indocyanine green (ICG) is the most popular imaging agent for image-guided surgery. IGC emits a light spectrum of 700-800 nm (a near-infrared fluorescent contrast agent), and has been approved by the US Food and Drug Administration for surgery.[11] It is cleared by the liver and has been used for a long time to evaluate the clearance function of the liver and to image blood vessels (angiography). After binding with plasma proteins, ICG is retained in tumor tissues through the increased permeability of tumor vessels and lymphatics.[28] In a trial of ICG, it was able to identify small metastatic tumors of hepatocellular carcinoma.[28] The fluorescent margin of the tumor was demarcated from surrounding normal tissues several hours to days after intravenous injection of ICG.[28] Many studies reported the clinical application of ICG for various solid tumors including colorectal, gastric, and head and neck cancer.[10,21,29,30] In addition to ICG, many fluorescent dyes, including Cy5.5, Cy7, IRdye800 CW, and quantum dots have been used for near-infrared imaging and image-guided surgery.[12,29]

## 4. Biologic markers for image-guided surgery

Even with a high tumor to background ratio, the above fluorescent imaging probes are not tumor-specific, accumulating in tumor tissues in a passive manner. Thus, several biological signals have been incorporated into molecular tumor imaging to enhance tumor-specific uptake intraoperatively. Folate receptor-alpha,[31] epidermal growth factor receptor,[32] HER2/neu,[33] prostate-specific membrane antigen,[34] transferrin receptor,[35] and carcinoembryonic antigen 19-9[36] have been studied to distinguish tumor from normal tissue in various types of cancer.

For example, in breast cancer, ICG with trastuzumab (monoclonal antibody against HER2 receptor) was investigated to visualize margins and to classify molecular subtype during surgery.[37] Adhesion molecule alpha-v-beta-3 integrin can also be an imaging target for tumor delineation by Cy5.5 or IRDye800 CW conjugate with an antibody against alpha-v-beta-3 integrin.[26,38,39]

A randomized controlled trial of 5-aminolevulinic acid (5-ALA) was a landmark study of an imaging probe that improved progression-free survival in glioblastoma patients.[40] However, this study did not provide preoperative molecular or genetic information regarding brain tumors with regard to whether cancer tissue had 5-ALA susceptibility.

The tumor environment is aberrantly transformed by cancer cells. This causes dysregulated extra- and intracellular pH,[41] hypoxia, [42] increased secretion matrix metalloproteinases,[43] cathepsins,[44] and g-glutamyl transpeptidase,[45] which are potential targets for tumor-specific image-guided surgery.

In head and neck cancers, near-infrared imaging dye of IRDye800 CW conjugated with anti-epidermal growth factor receptor

(panitumumab) has been used in head and neck squamous cell carcinoma.[46] In a phase I trial, cetuximab (anti EGFR antibody)-IRDye800 was shown to improve surgical resection more precisely with a high tumor to background ratio.[47] Tumor was sharply demarcated from surrounding normal tissues within 1 mm, and fluorescence was strongly correlated with the location of the tumor, as confirmed by biopsy.[48] Another study of cetuximab-IRDye800 for head and neck cancer also showed the safety and tumor specificity of image-guided surgery.[47] This study enrolled patients with biopsy-proven head and neck squamous cell carcinoma, not specified into positive epidermal growth factor tumor.

Transferrin receptor is a poor prognostic indicator and is overexpressed in head and neck cancer. An in vivo study showed that the maximum fluorescence signal of the tumors occurred between 90 and 120 min after injection and a high tumor to background ratio was achieved.[35]

Another study using Cy5.5 conjugated anti-vascular endothelial growth factor antibody (bevacizumab) in an animal experiment also demonstrated the feasibility of image-guided surgery for head and neck cancers using this probe.[49]

## 5. New definition of precision cancer surgery

Tumors may have different phenotypes and characteristics according to anatomical location, histologic subtype, previous treatment, tumor burden (stage) and chronological effect.[50] This can be a major hurdle to choosing the best-fit imaging probe that correctly or accurately visualizes target tumor cells in precision image-guided surgery. Thus, it should be further improved with a tailored method reflecting individual tumor-specific biological changes. In this proof of concept study, we suggest a new surgical concept, individual tumor biology-based (individualized) image-guided surgery, and investigate its potential significance in surgical oncology.

Traditionally, cancer treatment has been based on tumor location (primary site) and histopathology.[51] However, recent development of genomic and molecular technologies revealed that cancers from the same origin and histopathology can have molecular diversity, which may result in treatment resistance or failure.[52] With cancer genomic atlas, many clinicians and researchers expects that a rational selection of target agents for specific genetic and molecular alterations of cancer, even from the diverse origin sites.[52,53]

This would accelerate the feasibility of prevention, early detection, and patient-tailored treatment of various types of cancers. Translation of molecular and genomic data into cancer treatments enables personalized cancer medicine with specific target agents. [54]

Precision medicine in a basket clinical trial linked molecular targets specific to the genetic aberrations of cancer and target drugs, irrespective of the tumor origin.[55] Several genome- based cancer medicines have been used in clinical settings, including trastuzumab for HER2 mutation in breast cancer,[56] imatinib for tyrosine kinase KIT (CD117) aberration in gastrointestinal stromal tumors,[57] and erlotinib for epidermal growth factor receptor (EGFR) in lung cancer.[58] However, precision oncology does not promise clinical benefits; a previous report of 2,000 consecutive patients showed that only 6.4% of patients had actionable alterations fitting genotype-matched trials.[59] Nonetheless, rapid accumulation of genetic information, technology, and drugs has improved the clinical outcomes of several target agents.

The main purpose of cancer surgery is to identify the extent of tumor accurately and remove tumors completely with minimal loss of physiological function. In conventional oncological surgery, surgeons have relied on visual inspection with white light and tactile sensations during surgery, as mentioned before. Clinical application of intraoperative molecular imaging has improved the outcomes of surgical cancer treatments.[60] However, the imaging probes for intraoperative visualization of tumors are not tumor-

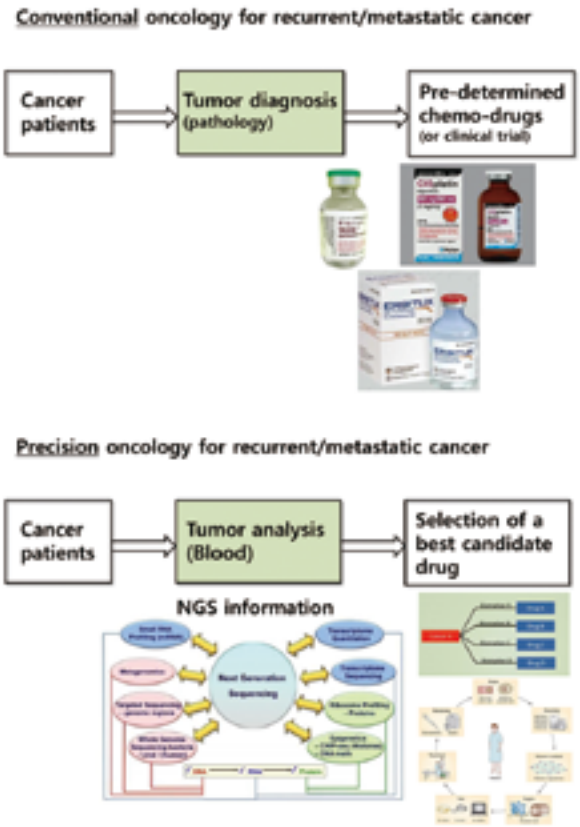


Fig. 1. Basic concept of precision oncology (precision medicine) compared with the conventional oncology.

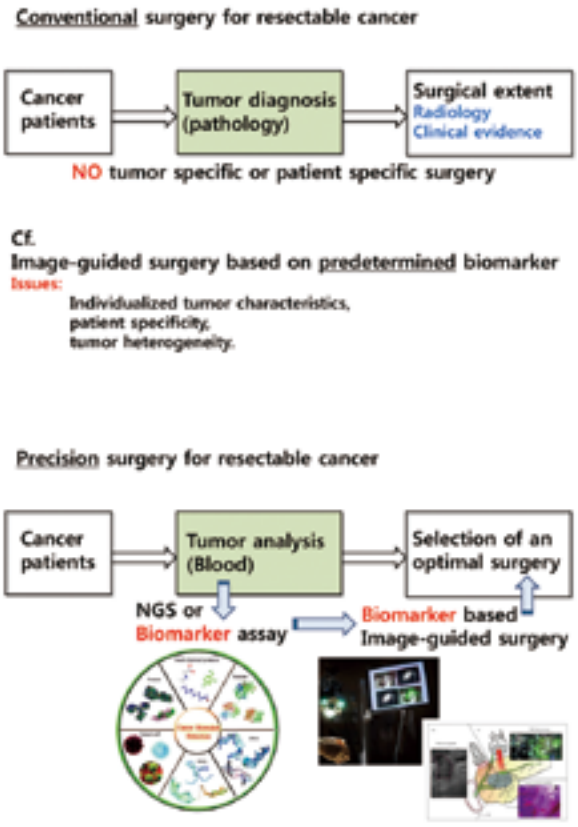


Fig. 2. Translation of a precision oncology concept to the precision cancer surgery In precision cancer surgery, tumors are analyzed molecularly and genetically to select the optimal imaging probes for individual tumors before surgical resection, beyond the use of predetermined imaging probes for certain types of cancer.

specific. As conventional oncology has moved toward precision oncology with genomic and biological information specific to each tumor, image-guided surgery should also shift toward tumor biology-based image-guided surgery, so-called precision surgery for cancer (Fig. 1 and 2). This is a simple concept with regard to more accurate image-guided surgery. In precision cancer surgery, tumors should be analyzed molecularly and genetically to select the optimal imaging probes for individual tumors before surgical resection, beyond the use of predetermined imaging probes for certain types of cancer.

This will raise the likelihood of meeting the surgical goals of cancer treatment. In short, precision cancer surgery can be defined as individual tumor biology-based image-guided surgery.

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# Image guided surgery in non-palpable breast lesions

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## 배 경

유방의 종양은 진단방법과 조기검진프로그램의 시행으로 영상검사에서 초기에 진단이 되었지만 수술적 치료 시 병변의 국소화가 어려워 정확한 부위를 수술하는 데에 여러가지 방법을 이용해왔다. 아직까지도 초음파로 병변 위의 피부에 표시하는 방법이나 guide wire를 삽입하거나 charcoal powder로 tattooing 등을 하는 방법이 널리 이용되고 있으나 정확도의 한계나 수술 후 채내에 오래 잔존하는 등의 단점을 가지고 있다. 이에 대한 발전된 형태로 2000년대에 들어서 radio-active seed를 병변에 삽입하여 감마카메라나 네비게이터로 수술 중에 추적하는 방법이 소개 되었고 근적외선 형광을 이용한 병변의 형광색소를 이용한 국소화방법도 시도 되었다. 2011년에는 Gooitzen 난소암의 복막 전이된 종양을 형광염료를 이용하여 종양의 folate-alpha receptor에 착색시키는데 성공하여 tumor specific intraop imaging으로 성공적인 선택적 절제를 성공했음을 Nature medicine에 보고 하기도 하였다.

이러한 흐름 하에 근적외선 형광을 이용한 image guided surgery는 유용성이 많이 보고 되고 있으나 국내에서 임상에 적용하기 위하여 기존의 방식 중 가장 정확도가 높은 Activates Charcoal tattoing과의 무작위 임상시험을 하게 되었다.

## 방 법

2018년 1월부터 2019년 3월까지 삼성서울병원과 명지병원을 방문하여 수술이 결정된 비촉지성 유방결절을 50명을 대상으로

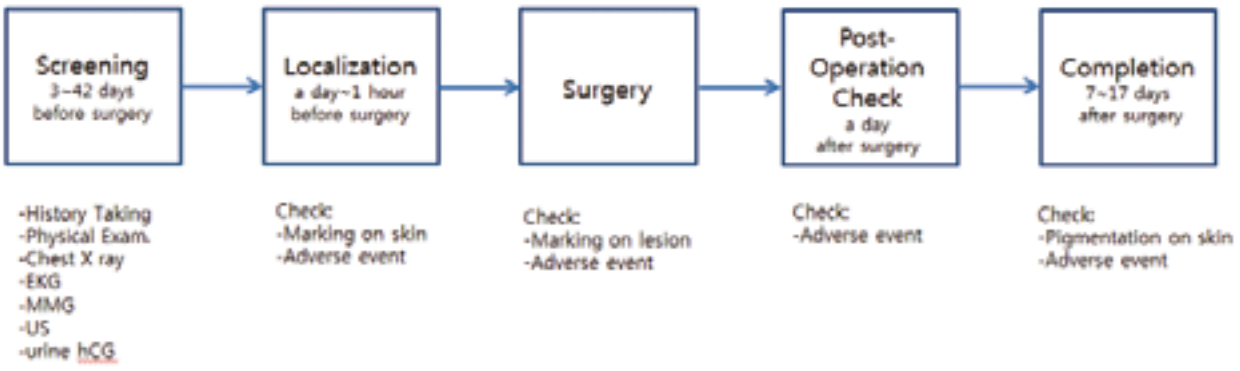


Fig. 1. 임상시험흐름도.

전향적, 무작위배정으로 ICG-hyaluronic acid mixture (LuminoMark)와 activated charcoal (Charcotrace)로 병변을 localization 후 수술을 시행하였으며 Luminomark는 NIR-fluorescence camera를 이용하여 수술을 진행하였으며 Charcotrace는 육안으로 수술을 진행하여 그 결과를 비교하였다(ClinicalTrials.gov Identifier: NCT03743259).

## 결 과

양군 간의 차이를 비교하였을 때 근적외선 형광을 이용한 방법이 절제완성도면에서 더 정확성을 보였으며 시술 후 피부침착이 남지 않는 차이를 보였다.

| | | Control group(n=14) | Test group 1(n=15) | Test group 2(n=15) | *p-value* |
|---|---|---|---|---|---|
| Age(yr) | <30 | 0 (0.0%) | 0 (0.0%) | 3 (20.0%) | *0.143* |
| | 30≤ <40 | 1 (7.1%) | 2 (13.3%) | 1 (6.7%) | |
| | 40≤ <50 | 5 (35.7%) | 9 (60.0%) | 7 (46.7%) | |
| | 50≤ | 8 (57.1%) | 4 (26.7%) | 4 (26.7%) | |
| | mean(range,yr) | 52.2(39-68) | 47.4(37-57) | 44.2 (26-76) | *0.112* |
| Pathology | Fibroadenoma | 4 (28.6%) | 6 (40.0%) | 7 (46.7%) | *0.384* |
| | Phyllodes tumor | 0 (0.0%) | 1 (6.7%) | 1 (6.7%) | |
| | Atypical ductal hyperplasia | 4 (28.6%) | 0 (0.0%) | 0 (0.0%) | |
| | Intraductal papilloma | 2 (14.3%) | 3 (20.0%) | 4 (26.7%) | |
| | Malignancy | 1 (7.1%) | 2 (13.3%) | 2 (13.2%) | |
| | Others | 3 (21.4%) | 3 (20.0%) | 1 (6.7%) | |
| the longest length on pre OP-US (cm) | <1 | 8 (57.1%) | 5 (33.3%) | 7 (46.7%) | *0.500* |
| | 1≤ <2 | 5 (35.7%) | 9 (60.0%) | 6 (40.0%) | |
| | 2≤ | 1 (7.1%) | 1 (6.7%) | 2 (13.3%) | |
| | mean(range,cm) | 1.0(0.4-2.7) | 1.2(0.6-2.8) | 1.3(0.6-2.7) | *0.379* |
| the longest length of excised specimen (cm) | <1 | 0 (0.0%) | 1 (6.7%) | 1 (6.7%) | *0.253* |
| | 1≤ <2 | 1 (7.1%) | 3 (20.0%) | 2 (13.3%) | |
| | 2≤ <3 | 6 (42.9%) | 7 (46.7%) | 7 (46.7%) | |
| | 3≤ <4 | 5 (35.7%) | 1 (6.7%) | 4 (26.7%) | |
| | 4≤ | 2 (14.3%) | 3 (20.0%) | 1 (6.7%) | |
| | mean(range,cm) | 3.0(1.5-8.0) | 2.4(0.8-4.0) | 2.5(0.6-6.2) | *0.247* |
| the longest length of pathological lesion (cm) | unknown | 3 (21.4%) | 2 (13.3%) | 4 (26.7%) | *0.791* |
| | <1 | 5 (35.7%) | 5 (33.3%) | 5 (33.3%) | |
| | 1≤ <2 | 4 (28.6%) | 8 (53.3%) | 5 (33.3%) | |
| | 2≤ <3 | 2 (14.3%) | 0 (0.0%) | 0 (0.0%) | |
| | 3≤ | 0 (0.0%) | 0 (0.0%) | 1 (6.7%) | |
| | mean(range,cm) | 0.9(0.5-2.7) | 0.8(0.5-1.7) | 0.8(0.5-3.0) | *0.894* |
| Marking on breast lesion | Yes | 13 (92.9%) | 13 (86.7%) | 14 (93.3%) | *0.954* |
| | No | 1 (7.1%) | 2 (13.3%) | 1 (6.7%) | |
| Marking on Excised specimen | Yes | 13 (92.9%) | 14 (93.3%) | 15 (100.0%) | *0.357* |
| | No | 1 (7.1%) | 1 (6.7%) | 0 (0.0%) | |
| Skin Pigmentation | Yes | 9 (64.3%) | 0 (0.0%) | 0 (0.0%) | *0.000* |
| | No | 4 (28.6%) | 15 (100.0%) | 15 (100.0%) | |
| | unknown | 1 (7.1%) | 0 (0.0%) | 0 (0.0%) | |
| The completeness of resection | mean (±sd) | 3.7(±1.5) | 2.2(±0.5) | 2.1(±0.5) | *0.026* |
| The pathological completeness | mean (±sd) | 0.3(±0.1) | 0.4(±0.1) | 0.4(±0.1) | *0.331* |

Fig. 2. 시험결과 비교표.

## 고 찰

기존의 방식에 비하여 근적외선 형광을 이용한 방법을 이용하면 좀더 정확한 병변의 국소화를 얻을 수 있으며 향후에 종양 별 specific target를 개발하여 tumor specific image guided surgery로 이어질 것으로 기대된다.

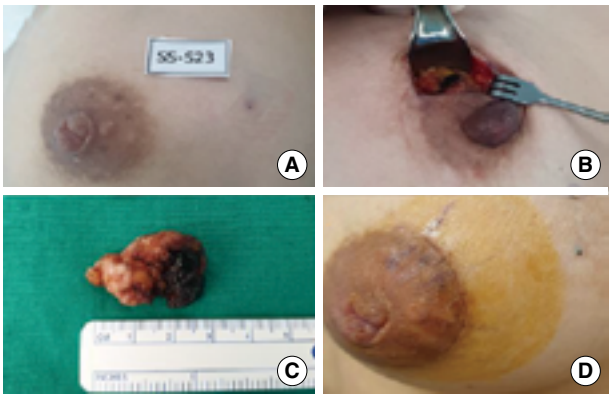


Fig. 3. Photos for patients in control group. (A) Before skin incision, (B) after skin incision, (C) after excision, (D) at last follow up day.

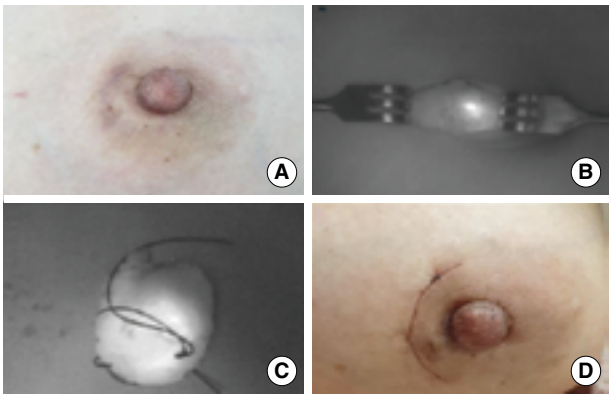


Fig. 4. Photos for patients in test group. (A) Before skin incision, (B) after skin incision, (C) after excision, (D) at last follow up day.

# 암 수술에 있어서 차세대 염기서열분석 (Next generation sequencing) 정보의 이용

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## 서론

차세대 염기서열 분석(Next generation sequencing, NGS)은 유전체 염기서열의 고속 분석 방법을 통칭하는 용어로[1] 기존의 생어 염기서열 분석(Sanger sequencing)과 달리 많은 수(백만 개 이상)의 DNA 조각을 병렬로 처리하는 데 특징이 있다.[2,3] 차세대 염기서열 분석의 등장으로 유전체 분석에 필요한 시간과 비용이 급격하게 줄어들어 많은 분야에서 다양하게 사용되고 있다.[4] 국내에서는 2017년 3월부터 NGS가 암환자와 희귀질환을 갖고있는 환자에서 보험이 적용되고 있는데 이는 NGS의 여러 가지 종류 중에서 targeted sequencing (TS)이 허용된 것으로 수십 개에서 수백 개의 특정 유전자를 짧은 시간 내에 적은 비용으로 염기서열을 분석해서 얻은 돌연변이 결과를 임상에서 사용하기 위함이다. 그러나 전체 유전체 염기서열 분석결과를 여러가지 생물정보학(bioinformatics)적 기술을 이용하여 분석하고 이를 임상에 적용하는 단계까지는 아직도 가야할 길이 멀기만 하다. 이 연제에서는 NGS의 기본 개념 및 종류, 암을 수술하는 외과의사의 관점에서 NGS 이용분야에 대하여 살펴보고자 한다.

## 차세대 염기서열 분석의 원리, 종류 및 특징

DNA 염기서열 분석법은 1977년 Sanger chain termination method가 도입되면서 가능하게 되었다.[5] Sanger방법을 이용해서 인간의 유전체 염기서열을 분석한 연구결과인 Human Genome Project가 Science, Nature에 15년에 걸친 연구 끝에 2001년 출판되었다.[6] 2000년대 중반 도입된 차세대 염기서열 분석법인 NGS는 기본적으로 유전체를 short read로 자른 후 massively parallel sequencing technique으로서 Sanger 방법과는 접근법이 근본적으로 다른 DNA 염기서열 분석법이다. Sanger 방법으로 한 사람의 유전체를 15년에 걸쳐서 거의 3조 원의 비용으로 분석했는데 지금은 NGS로 약 100만 원의 비용으로 하루만에 분석할 수 있는 시대가 되었다.

NGS와 Sanger 방법의 근본적인 차이는 Sanger 방법은 DNA single strand를 시퀀싱하지만, NGS는 하나의 긴 DNA strand를 수백만 개 가닥으로 자른 후 유전자 증폭을 하고 이를 한꺼번에 시퀀싱한다는 것이다. 이러한 NGS는 기본적으로 4단계로 구성되어 있다. 첫째, library preparation 단계로 DNA를 추출 후 무작위로 수백만 조각으로 낸 후 추후 PCR을 위해서 DNA 5'와 3'에 adapter를 붙인다. 둘째, cluster generation 단계로 DNA fragment 수백만 개 각각을 PCR을 통해 증폭시켜 DNA fragment의 clonal cluster를 만든다. 셋째, sequencing 단계로 DNA polymerase에 의해 DNA strand cluster에 상보적인 염기서열이 생성되면서 형광을 발현하게 되면 nucleotide 종류를 확인하게 된다. 넷째, 데이터 분석 단계로 생성된 수백만 개의 short read의 염기서열 데이터는 reference genome을 이용해서 앞뒤 순서를 배치하게 된다. 그후 bioinformatics를 통해서 염기서열 분석 결과를 얻게 된다.

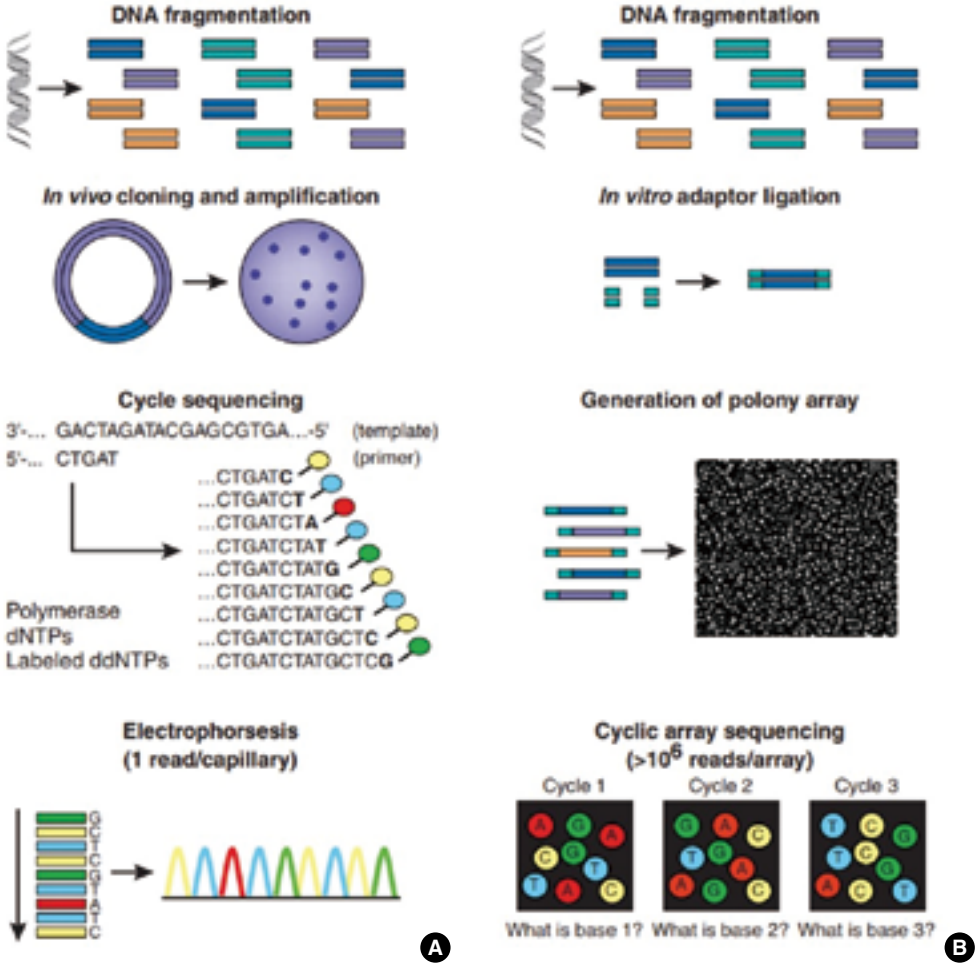


Fig. 1. Work flow of conventional versus next generation sequencing.[7]

Table 1. 여러가지 NGS platform의 특징

| 플랫폼 | 1가닥의 길이 | 정확도 | 1회 운영 시 가닥 수 | 1회 운영시간 | 100만 bp당 가격(미국달러) | 장점 | 단점 |
|---|---|---|---|---|---|---|---|
| 단일분자 실시간 염기서열분석 Single-molecule real-time sequencing | 10,000–15,000 bp | 87% | 50,000 | 30분–4시간 | 0.13–0.60 | 긴 가닥 길이로 빠른 분석 | 기계 가격이 비쌈 |
| 이온 반도체, 이온 토런트 염기서열분석 Ion semiconductor, Ion Torrent sequencing | 400 bp | 98% | 8천만 | 2시간 | 1 | 저렴한 기계가격, 빠른분석 | 연속된 염기서열 분석에 에러 발생 가능 |
| 파이로 시퀀싱, 454 염기서열 분석 Pyrosequencing, 454 technology | 700 bp | 99.9% | 백만 | 24시간 | 10 | 빠른 분석 | 가격이 비쌈 |
| 염기서열 합성분석 Sequencing by synthesis, Illumina | 50–300 bp | 99.9% | 25억 | 1–11일 | 0.05–0.15 | 정확한 염기서열, 가닥 산출 최대 | 기계 가격이 비싸 비 분석할 때 많은 DNA 요구 |
| 염기서열 묶음분석 Sequencing by ligation, SOLiD | 50+35 or 50+50 bp | 99.9% | 14억 | 1–2주 | 0.13 | 저렴한 가격 | 매우 느린 염기서열분석시간 |
| 나노포어 Nanopore Sequencing | 500bp | 92–97% | 사용시마다 다름 | 1분–48시간 | 500–1,000 | 매우 긴 가닥 휴대가능한 크기 | 저렴한 기계가격 낮은 정확도 |

## 암 수술에 있어서 차세대 염기서열 분석결과의 적용

### 1. Decision of treatment option: surgical vs medical

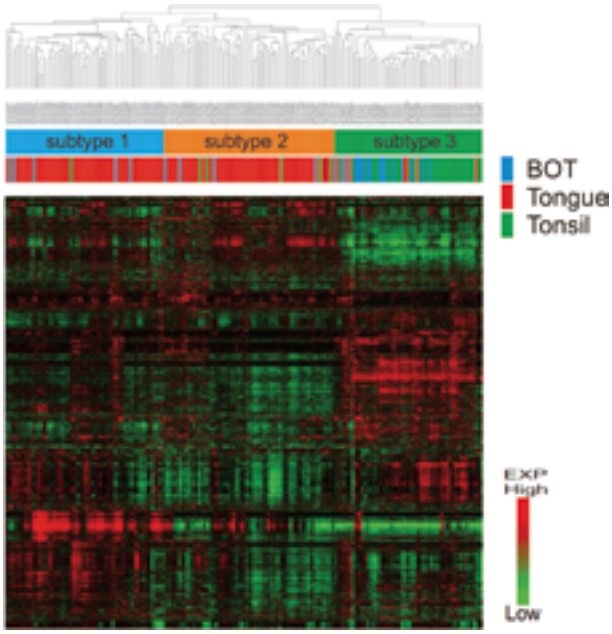


Fig. 2. Unsupervised cluster analysis with gene expression data for three different primary sites of head and neck squamous cell carcinoma in The Cancer Genome Atlas (n=193).

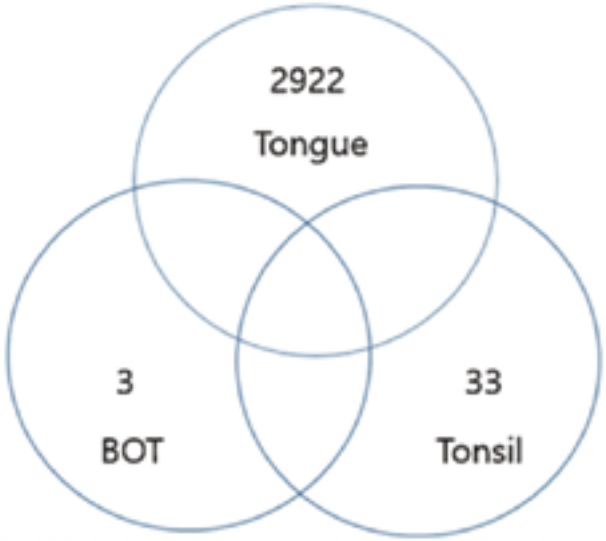


Fig. 3. Schematic diagram showing the number of primary site-specific genes for each site.

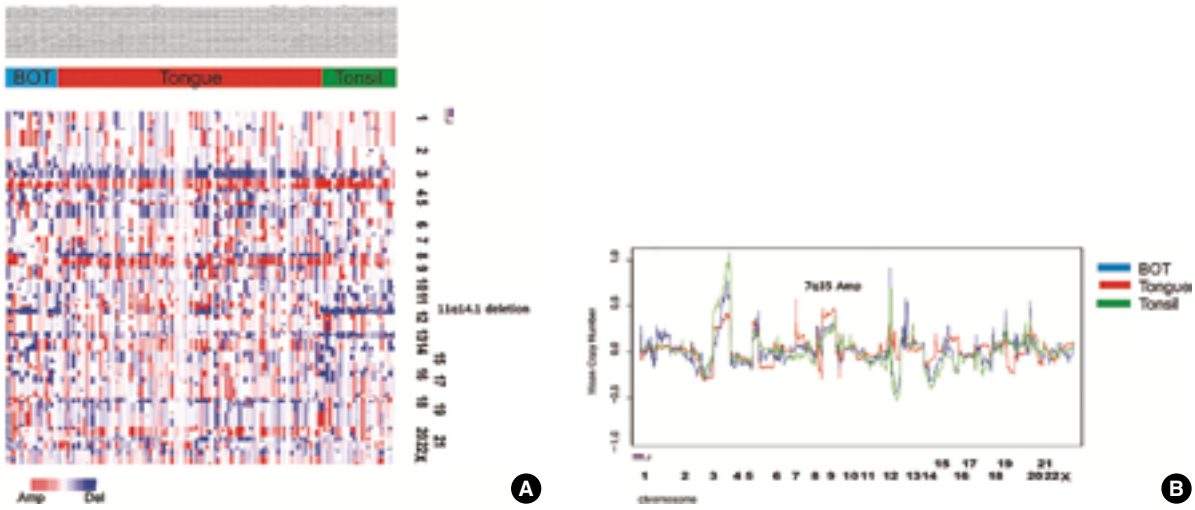


Fig. 4. Copy number (CPN) alterations analysis for head and neck squamous cell carcinoma at three primary sites: base of tongue (BOT), oral tongue, and tonsil. (A) The CPN alteration pattern of BOT cancer showed almost the same pattern as tonsil cancer, but not oral tongue cancer, (B)The pattern of CPN alteration shown on a linear graph.[8]

## 2. Extent of surgical resection: wide vs conservative

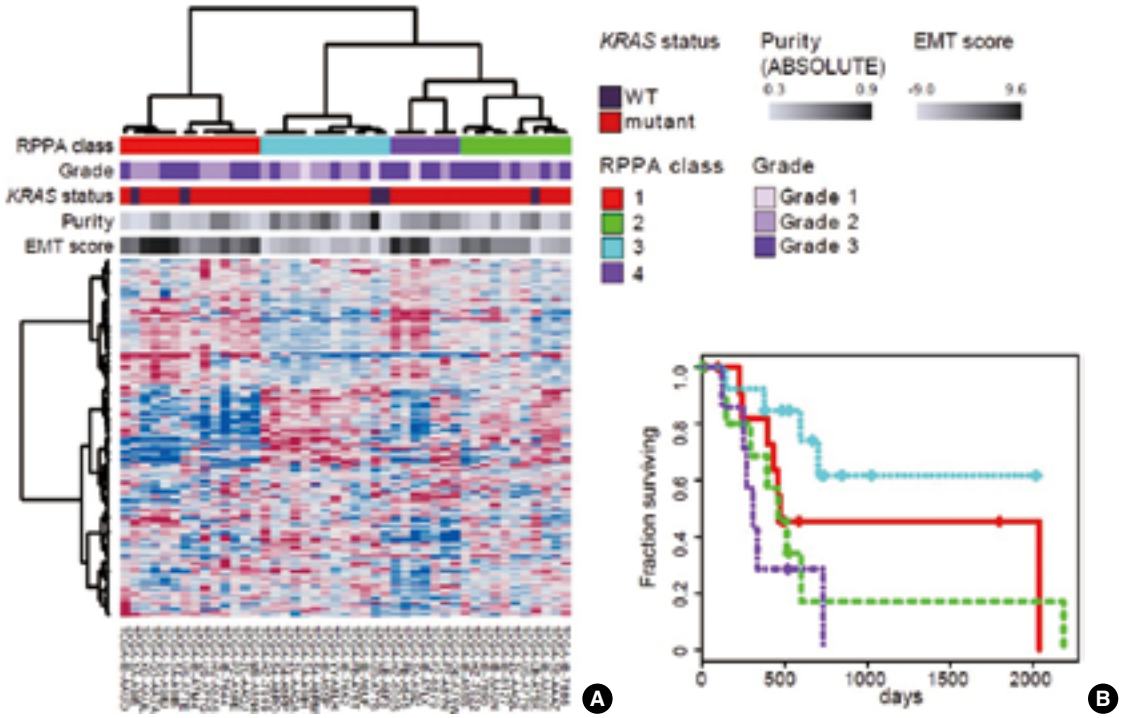


Fig. 4. RPPA Profiles Identify Biologically Distinct Subsets of High Purity Tumors. (A) Unsupervised consensus clustering of RPPA protein expression data for 45 of the 76 high-purity samples. (B) Cox survival analysis between clusters ($p$=0.045, likelihood ratio test from Cox analysis with purity as covariate).[9]

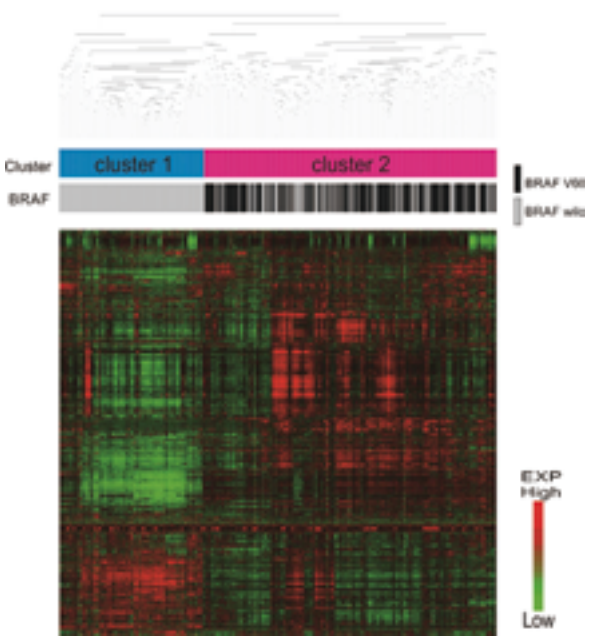


Fig. 5. Unsupervised clustering of papillary thyroid carcinomas (PTCs) revealing 2 distinct molecular subtypes.

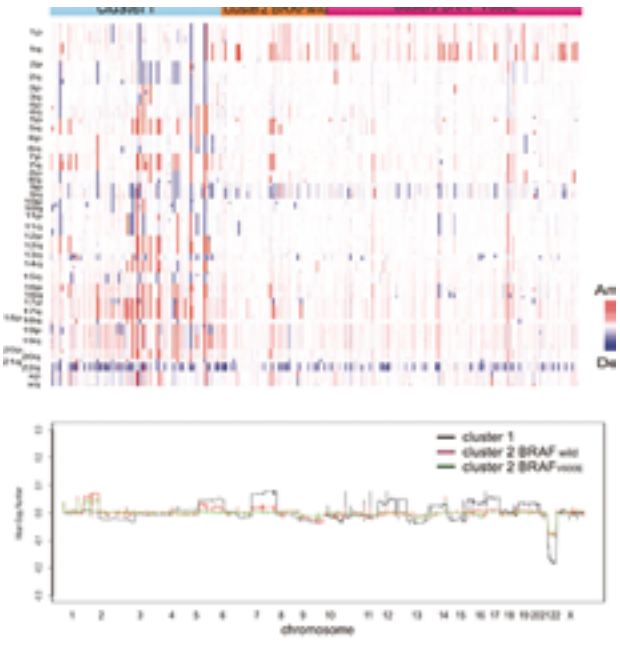


Fig. 6. Copy number alterations according to each group. The amount of copy number variations is small (20.2 to10.1) when compared with other cancer types.[10]

### 3. Extent of neck dissection

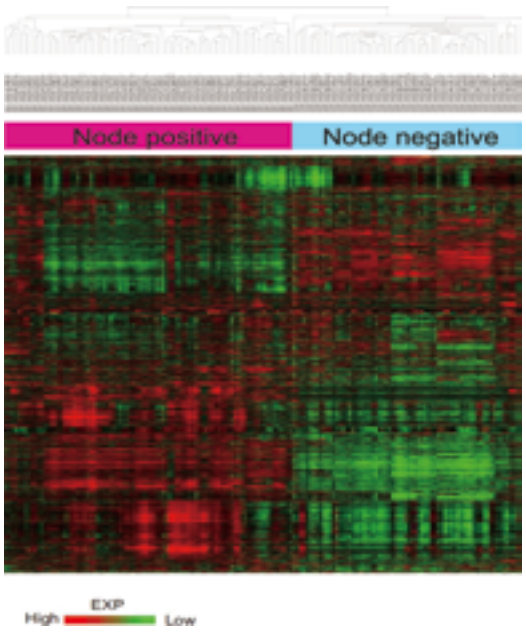


Fig. 7. Unsupervised clustering heat-map showing two distinct molecular patterns of gene expression between PTCs with nodal metastasis and PTCs without nodal metastasis.

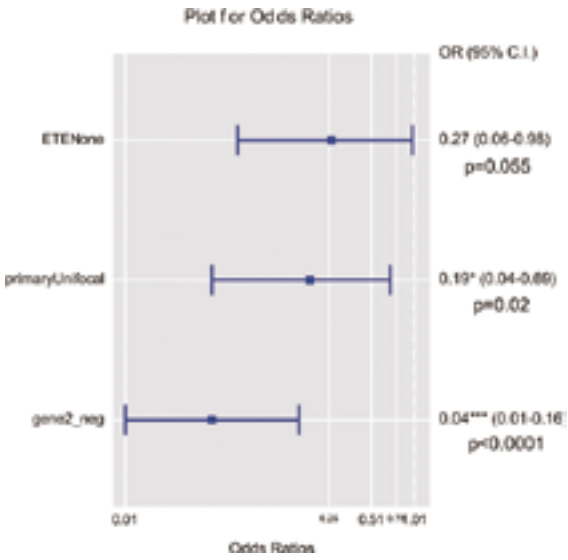


Fig. 8. Plot for odds ratios of each variable in validation cohort. Logistic regression with univariate analysis revealed that ETE, multifocality, and 12-gene signatures were significantly correlated with nodal metastasis. Multivariate analysis revealed that these 12 predictive gene signatures had significantly higher odds ratio than other variables.[11]

### 4. Molecular subtypes: considering post-operative CCRT

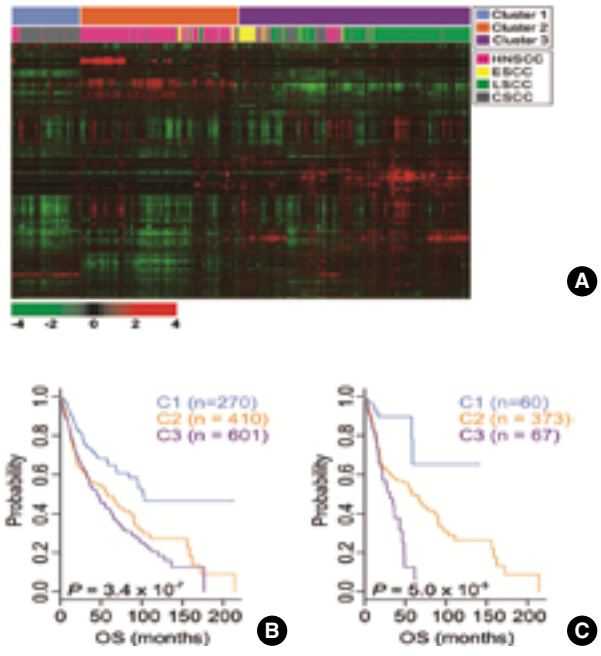


Fig. 9. Cluster analysis with gene expression data from four different types of squamous cell carcinoma (SCC): Head and neck SCC (HNSCC), esophageal SCC (ESCC), lung SCC (LSCC), and cervical SCC (CSCC).

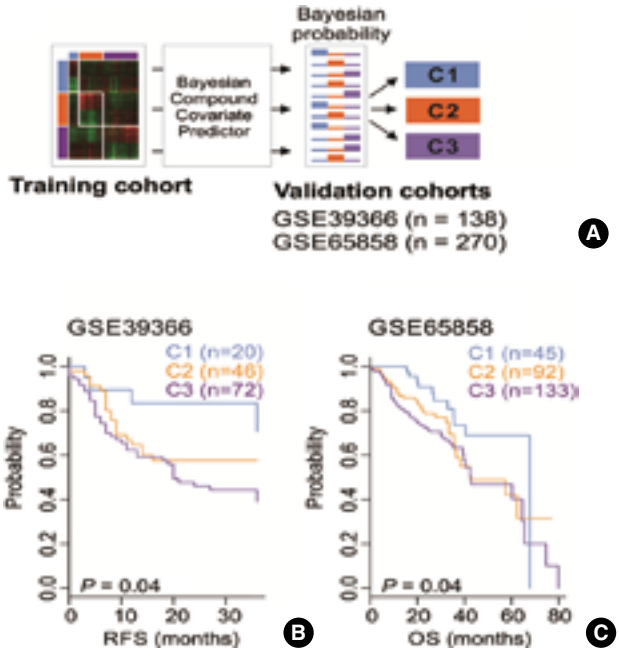


Fig. 10. Prognostic association of the three subtypes in two independent validation cohorts of patients with head and neck squamous cell carcinoma.

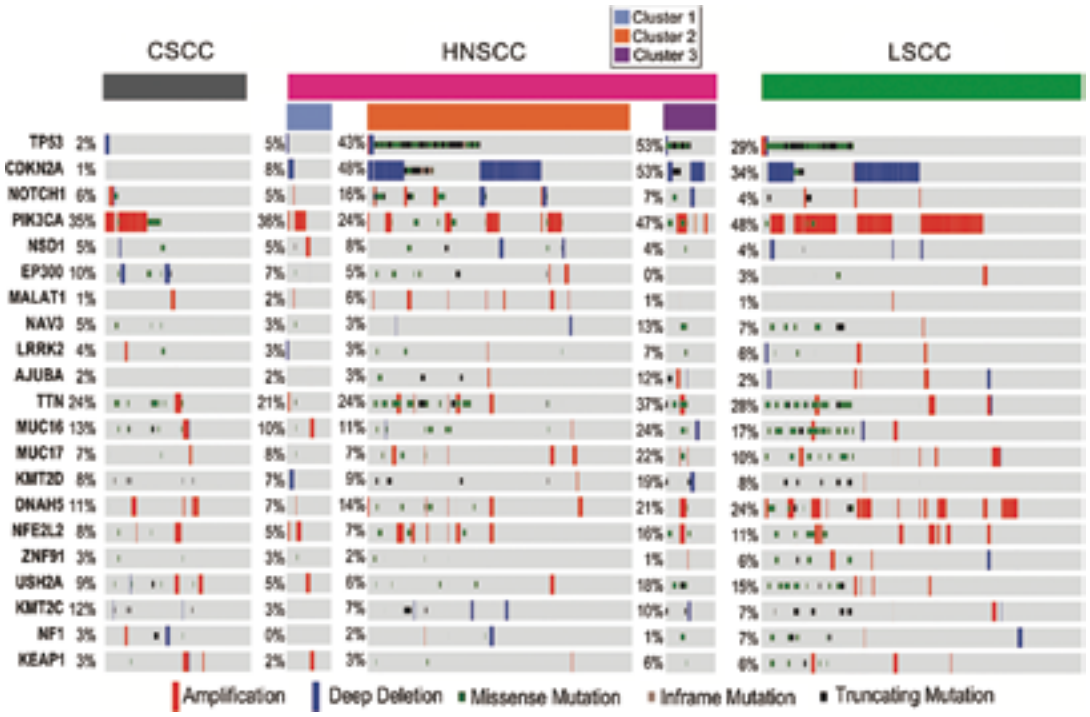


Fig. 11. Most frequently altered genes in head and neck squamous cell carcinoma (HNSCC) compared with cervical SCC (CSCC) and lung SCC (LSCC).[12]

## 맺음말

암 수술에 있어서 NGS는 1) 암을 치료하는데 있어서 수술적 치료를 선택할지 항암방사선 치료를 선택할지를 결정하는데 도움을 줄 수 있으며, 2) 암의 aggressiveness vs indolence 정도에 따라 수술적 절제 범위를 광범위하게 혹은 비교적 협소하게 시행하는데 있어서 정보를 제공하며, 3) 림프절 전이 등을 예측함으로써 림프절 절제술의 유무 혹은 범위를 결정할 수 있으며, 4) 분자생물학적 아형(molecular subtype)을 결정하여 이에 대한 적절한 수술 후 치료방법을 결정하는데 도움을 줄 수 있다.

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# Surgery combined with immunotherapy

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## 서 론

고형암의 치료에 있어서 환자의 병기가 국소절제술로 제거가 가능한 종양인 경우 가장 오래되었으면서도 아직까지 가장 효과적인 방법은 수술이다. 하지만 암을 가진 모든 환자의 절반만이 근치를 목적으로 수술을 받고, 상당수의 재발은 원격 전이로 인한 것이다. 따라서 수술적 치료의 보조적 요법으로 술후보조(adjuvant) 또는 술전신보조(neoadjuvant) 치료를 시행하게 된다. 세포독성T림프구관련 단백질 4 (cytotoxic T-lymphocyte–associated protein 4, CTLA4)와 프로그램세포사멸 1 (programmed cell death 1, PD-1) 및 프로그램세포사멸리간드 1 (programmed cell death 1 ligand, PD-L1)을 표적으로 하는 항암면역요법은 장기적으로 보았을 때 기존에 중심적인 역할을 해왔던 수술과 더불어 고전적인 방사선치료(radiotherapy), 항암화학요법(chemotherapy)과 함께 새로운 대안이 될 수 있는 혁신적인 치료법이다.

## 항암면역요법(Immunotherapy)

프로그램세포사멸 1 (PD-1) 경로를 차단하는 것을 기본으로 하는 항암면역요법은 암 치료에 있어 가히 혁신적이라고 할 수 있다. 현재까지 미국식품의약품안전청(FDA)은 10종류의 암에 대해 PD-1 억제제를 승인하였다. 이 약물의 치료 효능은 PD-1의 음성신호전달로 억제된 내인성 종양 항원 특이성 T 세포(endogenous tumor-antigen-specific T cells)의 면역관문(checkpoint)를 풀어주는 것이다. 전임상모델 및 인체에서의 바이오마커를 이용한 연구의 결과에 따르면 종양미세환경(tumor microenvironment)으로 모집되고 기능적으로 억제되는 항종양(antitumor) CD8+T 세포가 치료 효과의 중요한 역할을 한다는 것이 밝혀져 있다. PD-1 경로를 차단함으로써 정상적으로 작동하는 기능성T 세포가 증가하고 결과적으로 종양 사멸을 유도한다.

특히 재발되거나 전이된 암종(recurrent and/or metastatic, R/M)에서 종양의 크기를 줄이고 장기간 유지하거나 완치까지 보이는 사례가 보고되고 있어 향후 십수년 이내에는 이러한 진행된 경우를 치료하는 데 사용되겠지만, 이후에는 수술적 치료가 가능한 조기암에서도 예상치 않은 재발이나 전이를 줄이기 위해 점차 적용이 될 것이다. 따라서 항암면역요법과 수술을 어떻게 잘 병행하는 것이 치료에 도움이 되는지 알아보는 것은 그 의의가 매우 크다고 할 수 있다.

## 항암면역요법과 수술(Surgery combined with Immunotherapy)

술전신보조요법(neoadjuvant therapy)은 근치적 수술 전에 암에 대한 전신적치료(systemic therapy)를 의미한다. 수술 전후 전신

요법의 주요 목적은 일차 수술 방법이 기술적으로 어려운 환자에서 수술이 가능하게 하거나 용이하게 하는 것과 더불어 근치가 가능한 종양일지라도 수술 당시 이미 있을지 모르는 미세 전이 병소를 사전에 사멸시켜 수술 후 전신전이를 줄이고자 하는 의도도 가지고 있다. 전신 전이 위험이 있는 두경부암을 포함한 많은 고형암종에서 전신적치료(systemic therapy)를 조기에 시작함으로써 생존율이 향상될 것이라는 가설이 오래동안 있어왔지만, 고전적인 항암제를 이용한 방법으로는 그 가설이 실제로 입증된 예는 많지 않다. 흑색종, 비소세포폐암(NSCLC) 및 신세포암종(RCC)을 포함한 진행성 전이성 악성 종양의 치료를 위해 면역치료제인 CTLA4와 항PD-1 /PD-L1이 FDA 승인을 받았지만, 이들 약제들은 현재 술후보조요법(adjuvant) 조건에서 효능을 평가 중이다. 절제가능한 III기 흑색종에서 면역항암제인 CTLA4를 보조항암요법으로 사용한 무작위 3상 임상시험에서 면역항암제의 보조항암요법이 위약에 비해 무진행생존율 relapse free survival이 개선되었다는 보고가 있지만 그 결과를 아직 완전이 인정하기는 이르다. 한 연구에서는 절제가능하고 수술을 받은 흑색종 IIIC기 및 IV기 환자에서 술후보조요법으로 항 PD-1 백신을 투여 한 결과 무병생존율이 개선된 것으로 나타났다. 항암면역요법은 그 치료 효과에 대한 기대가 큰 바, 항PD-1 등의 약제를 술전신보조요법에서 사용하려는 시도들도 진행되고 있다. 임상적으로 표적치료 및 항암면역요법(ipilimumab)을 포함한 술전신보조요법이 다양한 고형종양의 치료 성적을 개선시킨다는 보고들이 있다. 하지만 흑색종에서는 일대일 비교 연구가 최근 개설된 바 있지만 술전신보조요법이 술후보조요법과 비교하여 더 효과가 있는지 여부는 알려져 있지 않다.

## 항암면역요법과 수술의 근거(Rationale for Surgery combined with Immunotherapy)

종양에 대한 근치적 수술이 인체의 면역체계를 저해한다는 간접적인 증거들이 존재한다. 이러한 효과는 종양에 대한 외과적 절제술 후 며칠에서 몇 주간 지속되며, 이러한 휴면 중인 종양의 증식과 원격 전이를 가능하게 하는 "면역억제의 창(immunosuppressive window)"이 존재하는 것으로 알려져 있다. 수술 직후의 시기는 애초의 원발 종양의 발달 및 진행 기간에 비하면 훨씬 짧은 기간이다. 하지만 최근의 연구들은 수술 후 유도된 이 짧은 면역억제의 기간이 수술 후 전이가 이루어지는데 결정적인 기간임을 보여준다. 따라서 이는 수술전후 항암면역요법을 사용하는 근거가 될 수 있다.

## 두경부암에서 수술과 항암면역요법(Immunotherapy and Head & neck surgery)

현재 두경부암에 대해서는 새로 진단받은 치료 경험이 없는 대상자에 대해 술전항암면역요법(adjuvant immunotherapy)으로 (Pembrolizumab)을 이용한 무작위, 활성 제어, 공개 라벨 연구(MK-3475-689)가 진행중이다.

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# Reconstruction through tissue engineering

권성근

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## Flap

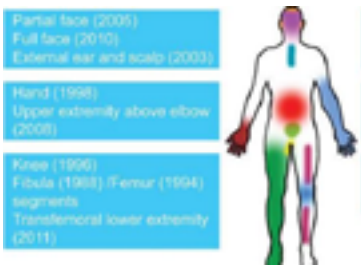
- Extensive tissue defects due to
  - Trauma
  - Tumor resection
  - Genetic and/or chronic diseases
  - Excessive debridement

→ Traditional reconstructive techniques with autologous composite tissue transfers
  - ✓ Flaps

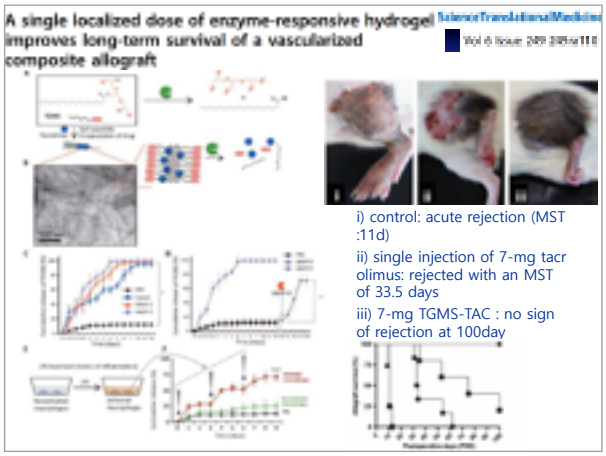
## Drawbacks of Flap

- Increased operative time
- Complexity
- Cost
- Limited availability of qualitative autologous tissue
- Wound healing complications
- Tissue flap failure
- Substantial donor-site morbidity.

## Vascularized Allograft



| Immunosuppressant | Mechanism of Action | Systemic Side Effects |
|---|---|---|
| Tacrolimus (FK506) | Calcineurin Inhibitor | Nephrotoxic, neurotoxic, hypertension, glucose intolerance, hyperkalemia, metabolic acidosis, hyperlipidemia |
| Corticosteroids i.e. prednisone | Inhibits production of IL-1 and IL-6; blocks T-cell activation | Osteonecrosis, osteoporosis, CNS effects, growth supression, glucose intolerance, hypertension, obesity, poor wound healing, adrenal suppression, cataracts, cushingoid features |
| Cyclosporine | Calcineurin Inhibitor | Nephro- & neurotoxic, hypertension, tremor, excess hair growth, gingival hyperplasia, glucose intolerance, hyperkalemia, metabolic acidosis, hyperlipidemia |
| Azathioprine | Inhibits purine synthesis | Bone marrow depression, hepatotoxic, posttransplant lymphoproliferative disorder (PTLD) |
| Mycophenolate Mofetil | Impairs B- and T-cell proliferation | Gastrointestinal toxicity (abdominal pain, nausea, vomiting, diarrhea), leukopenia, myelosuppression, viral infections, spontaneous abortions |
| Sirolimus | mTOR Inhibitor; inhibits T-cell proliferation | Hypertension, myelosuppression, diarrhea, proteinuria, poor wound healing, skin rash |

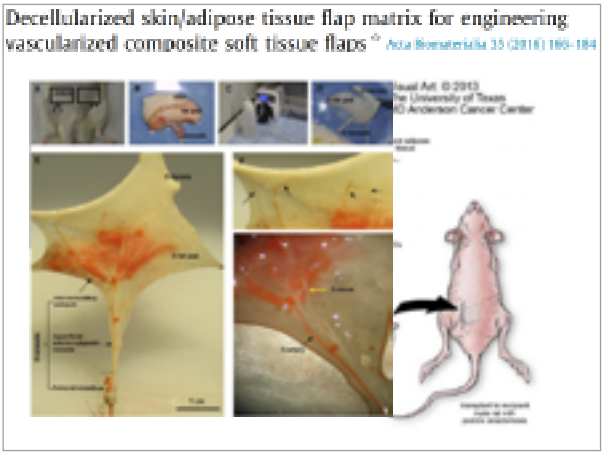


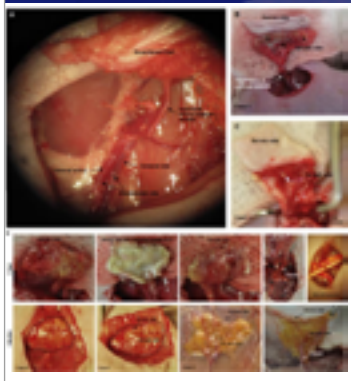

## Bioengineering Composite Tissue Flaps

- "ready-to-use-off-the-shelf" composite tissue flaps that are biologically active meanwhile being readily accepted by the host immune system
- Decellularized Tissue Scaffolds
- Synthetic Tissue Scaffolds
- Three-dimensional (3D) Bioprinting
- The Vascularization Problem
  - Engineering Vascular Grafts
  - Engineering Vascularized Networks and In-Situ Recruitment of Endogenous Vasculature

## Decellularization

- taking cadaveric organs or tissues, decellularizing them to leave only the extracellular matrix, and then repopulating the matrix with a patient's own autologous stem cells.
  → engineered tissue flap that architecturally was constructed with the scaffold from a cadaver but contains the patient's own cells to circumvent the risk of immune rejection
- applying chemical, physical, and enzymatic techniques



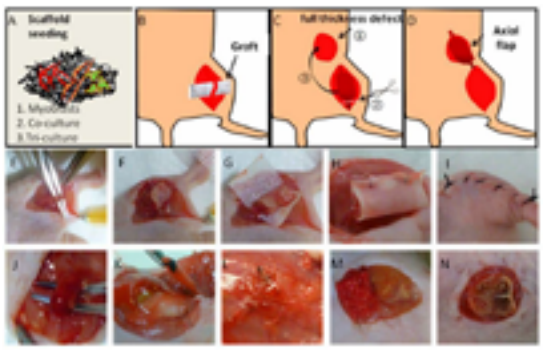



not restricted by the extent of the flap because it could be perfused instantly through microanastamosis from the host's vessels.
→ decellularized grafts coupled with recellularization show promise for the creation of large composite tissue flaps for reconstruction

## Synthetic scaffold

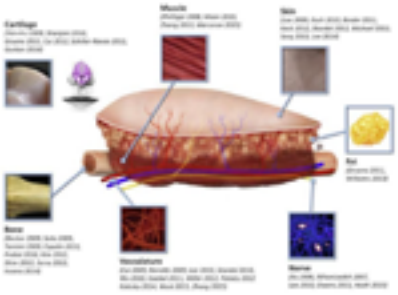
- The major advantages over decellularized scaffolds
  - more readily available, as they do not require donor tissue
  - can be made freely in the laboratory
  - there is less batch-to-batch variability
  - they are amenable to commercial scale up
  - can be modified much more extensively in shape, si ze, and function

## An engineered muscle flap for reconstruction of large soft tissue defects



## Three-dimensional (3D) Bioprinting

- a novel technology able to design and produce tissue- specific co nstructs by creating complicated, heterocellular structures with mi cro-scale precision
- physiological testing capabilities and reconstruction purposes



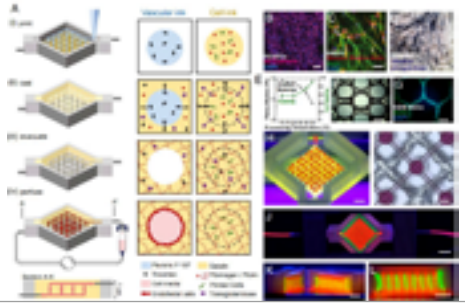
## Three-dimensional (3D) Bioprinting

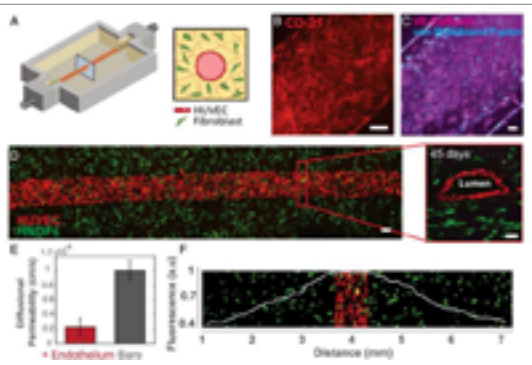
- Criticism
  - its short survival times
  - being limited to thin-tissue production
  - inability to recapitulate complex, composite tissues

## Three-dimensional bioprinting of thick vascularized tissues

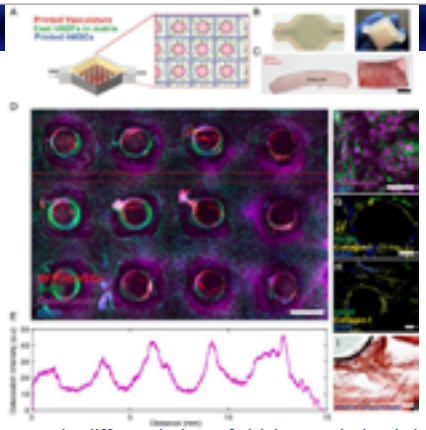
PNAS | March 22, 2016 vol. 113 | no. 12 | 3179–3184

- thick human tissues (>1 cm) with an engineered ECM, embedded vasculature, and multiple cell types
- perfuse 3D tissues that integrate parenchyma, stroma, and endoth elium





- Three-dimensional vascularized tissues remain stable d uring long-term perfusion



❖ Osteogenic differentiation of thick vascularized tissue

## The Vascularization Problem

❖ lack of biologically-realistic blood flow into engineered and printed tissue Phelps et al., Engineering more than a cell: vascularization strategies in tissue engineering. Current Opinion in Biotechnology, 2010. 21(5): p. 704-709

❖ The maximum distance between cells and capillaries is no longer than 200 μm in distance, which corresponds also to the diffusion limit of oxygen Kannan, et al., The roles of tissue engineering and vascularisation in the development of micro-vascular networks: a review. Biomaterials, 2005. 26(14): p.1857-1875

## Two strategies

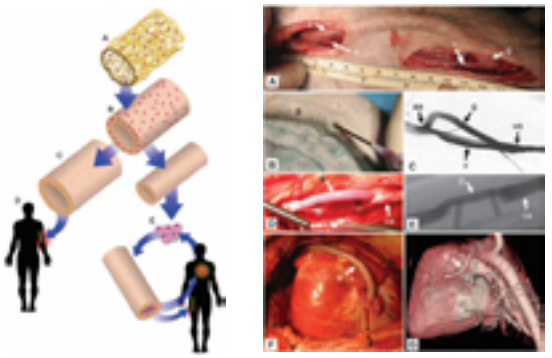
❖ development of complex, tissue engineered vascular networks
→ in vitro perfusion supports in vitro viability
→implantation in the in vivo environment results in delayed integration, angiogenesis, and vasculogenesis that cause ischemia and reduce cell viability.

❖ in situ recruitment of endogenous vasculature /or progenitor cells into a wound site or onto acellular tissue engineered scaffolds to re-establish vascular networks

## optimal vascular graft

❖ Mechanical strength
❖ High compliance to confront prolonged hemodynamic insults
❖ Immunotolerance
❖ Biocompatibility, differing sizes
❖ Suturability and effortless handling
❖ Thrombosis resistant
❖ Capable to defy infection
❖ Complete incorporation into the recipient bed
❖ Reasonable manufacturing costs

Readily Available Tissue-Engineered Vascular Grafts



## Summary

❖ Clinicians should be aware of new technologies and should be on the forefront in helping basic scientists move promising technologies from the bench to the bedside through clinical development.

❖ Many of these technologies have the potential to decrease operative time, meaning that surgeons may be able to provide medical care to more patients and in the meantime, provide better individual care due to reduced donor-site morbidity


Thank you for your attention
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