Table 5 Result of budget impact estimation

1. Annual cost per case

		First year	Second year	Third year	Fourth year	Fifth year
NCCN[a]-guided treatment vs. RS[b]-guided treatment	NCCN-guided treatment	¥1,677,915	¥535,596	¥541,683	¥548,444	¥579,241
	RS-guided treatment	¥1,976,790	¥536,596	¥542,448	¥548,958	¥579,614
St Gallen-guided treatment vs. RS-guided treatment	St Gallen-guided treatment	¥1,657,096	¥536,627	¥543,647	¥551,397	¥582,994
	RS-guided treatment	¥2,002,128	¥536,594	¥542,439	¥548,939	¥579,581

2. Annual incidence

	2008	2009	2010	2011	2012
Incidence of breast cancer	43,939	44,754	45,569	46,150	46,731
Incidence of LN−, ER+, ESBC	12,610	12,844	13,078	13,245	13,412

3. Budget impact estimation

		2008	2009	2010	2011	2012
NCCN-guided treatment vs. RS-guided treatment	Cost of NCCN-guided treatment	¥21,158 million	¥28,274 million	¥35,572 million	¥42,937 million	¥50,733 million
	Cost of RS-guided treatment	¥24,927 million	¥32,140 million	¥39,553 million	¥46,972 million	¥54,844 million
	Incremental cost	¥3,769 million	¥3,866 million	¥3,961 million	¥4,035 million	¥4,111 million
	Budget impact	¥2,638 million	¥2,706 million	¥2,773 million	¥2,825 million	¥2,877 million
St Gallen-guided treatment vs. RS-guided treatment	Cost of St Gallen-guided treatment	¥20,856 million	¥28,025 million	¥35,346 million	¥42,743 million	¥50,576 million
	Cost of RS-guided treatment	¥25,247 million	¥32,465 million	¥39,845 million	¥47,307 million	¥55,183 million
	Incremental cost	¥4,351 million	¥4,440 million	¥4,518 million	¥4,546 million	¥4,607 million
	Budget impact	¥3,046 million	¥3,108 million	¥3,163 million	¥3,195 million	¥3,225 million

[a] National Comprehensive Cancer Network

[b] Recurrence Score

high price of the 21-gene RT-PCR assay is not cancelled out by the reduction of adjuvant chemotherapy.

Costs treating LN−, ER+, ESBC incidence with NCCN/St Gallen/RS-guided treatment are calculated by the year taking mortality into account, and incremental costs are also calculated by the year according to comparisons. Calculated with these costs, the budget impact of the diffusion of the assay in Japan is estimated as ¥2,638 million (US$23 million) to ¥3,225 million (US$28 million).

Discussion

We evaluate the cost-effectiveness of the 21-gene RT-PCR assay in Japan's health care system with two scenarios depicting status quo and one scenario of the routine use of the assay for LN−, ER+, ESBC. Our economic model indicates that the diffusion of the assay gains more in terms of outcome but costs more at the same time. The estimated ICERs, 2,997,495 ¥/QALY (26,065 US$/QALY) and 1,239,055 ¥/QALY (10,774 US$/QALY), comparing NCCN/St Gallen-guided treatment with RS-guided treatment, respectively, are not more than a suggested social willingness-to-pay for one life year gain from an innovative medical intervention in Japan, 6,000,000 ¥/QALY (52,174 US$/QALY) [36]. Sensitivity analyses show that this result is plausibly robust, since ICERs do not exceed the threshold by various changes of assumptions made or values employed. In this sense, the assay has good value for money.

Incremental effects in terms of QALY are longer than those in terms of YOLS; and ICERs in terms of yen per QALY are smaller than those in terms of yen per YOLS in both comparisons. These imply that the assay is not only efficient in prolonging survival but also improving quality of life.

Our sensitivity analyses also reveal that the price of the assay is one of the major determinants of cost-effectiveness as expected. An intuitive comparison with the price of a conventional gene diagnosis test of malignant tumour in Japan, ¥450,000 (US$3,913) vs. ¥20,000 (US$174), seems to make a health manager feel it difficult to reimburse the cost of the assay by the social insurance, because there may be an incompatibility to an incremental manner of revising fee schedule. Our study, however, implies that the price offered by Japanese supplier of Onco*type* DX® Breast Cancer Assay still makes ICER an acceptable level from the viewpoint of welfare economics.

We estimate the budget impact of the assay on the social health insurance system. The policy implication of the budget impact is not prescriptive [37]. Yet, the estimated impact, ¥2,638 million (US$23 million) to ¥3,225 million (US$28 million) per year for the coming 5 years, is substantially less than the estimated budget impact of adjuvant trastuzumab, which is about to be included into social insurance benefit, ¥16,000 million (US$139 million) to ¥32,000 million (US$278 million) [38]. The characteristics of the assay of which application is limited to only once per case probably contribute to this difference, since the cost of trastuzumab amounts through its repeated administration. This implies that the diffusion of the assay through listing as an approved diagnostic test by the social health insurance could be justifiable.

The past economic evaluation of the assay reported from the U.S. considers a change from NCCN-guided treatment to RS-guided treatment [19], while our model allows a comparison between NCCN-guided treatment and St Gallen-guided treatment as an ex ante scenario. We find a notable difference in ICERs in this comparison. The ICER of the change from St Gallen-guided treatment is more favourable than that from NCCN-guided treatment. This is interesting because the reduction of use of adjuvant chemotherapy according to the reclassification from St Gallen criteria, 26%, is smaller than that from NCCN, 43%. The difference in ICER is due to more gain in the outcome. Although caution is needed in transferring the findings from economic models to any different context [39], our model might indicate that the assay has better value for money in countries where St Gallen-guided treatment is widely used.

However, this study has its own limitations. First, our outcome estimation depends on the validation studies carried out in the U.S. Although the evidences adopted are considered as the best available knowledge, it is needless to say that there are differences in population, as well as in cancer care practice between the U.S. and Japan. With this in regard, another validation study employing Japanese historical clinical trial data with the gene assay of preserved tumour tissue is launched [40]. A further economic evaluation incorporating new evidences is necessary to confirm the findings of this study. Second, utility weights adopted here are also derived from Western countries due to an unavailability of data from Japan. Third, our model does not include potentially costly clinical stages such as local recurrence or contralateral breast cancer due to the lack of data in validation studies. Regarding these shortcomings, reports and data that refines the model are awaited. Fourth, consensus guidelines are renewed continuously by incorporating newly available evidences [11, 41], so that the relative usefulness of the assay may be diminished in the near future, or the assay may be incorporated in the guidelines in a long run.

The use of the 21-gene RT-PCR assay has just begun to have an impact on clinical recommendations made by the U.S. oncologists and patients' choice [42]. It is easy to imagine that similar change in practice will occur in Japan

soon, because patients have strong preference to innovation such as tailor-made medicine [1]. As the prognostic usefulness of the 21-gene RT-PCR assay in guiding treatment for lymph-node-positive cases is recently reported [43], the indication of the assay will expand. Further economic evaluation that responds to this contextual change may become imperative.

Once the usefulness of the assay is confirmed by the Japanese validation study, Japanese health manager inevitably needs to decide how to fit the assay to the health care system. The results of this study imply the possibility of coverage by the social insurance. If health manager gives much importance to fiscal policy or cost containment, the selective indication of the assay for higher risk patients, which results to avoid additional use of adjuvant chemotherapy, might be a potential option. Further analysis incorporating such scenarios may be useful.

In conclusion, the routine use of the 21-gene RT-PCR assay for LN−, ER+, ESBC is indicated as cost-effective with a fundable level of budget impact in Japan. The results could inform health managers in developed countries where NCCN-guided treatment as well as St Gallen-guided treatment are practiced.

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特別附録

P07-039

乳癌高リスク者によるホルモン療法剤予防内服の費用効果分析

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【目的】乳癌の高リスク者によるホルモン療法剤タモキシフェン(TAM)の予防内服が発生率を低下させると複数の臨床試験で示され、欧米では予防内服が臨床で行われている。またラロキシフェン(RAL)の有効性も臨床試験で示された。我が国の癌医療を巡っては欧米で利用可能な選択肢を時期を失することなく国民に利用可能にすることを求める声が高まっているが、現行の日本の医療・公衆衛生制度のもとでは薬剤内服による癌予防を行うことはできない。予防内服という選択肢の導入を考えるには効率性を検討することも重要であると考えられる。本研究はTAMおよびRAL予防内服の費用対効果を明らかにすることを目的とした。【方法】NSABP P-1 study (*J Natl Cancer Inst.* 2005 97(22):1652-62.) および NSABP P-2 study (*JAMA.* 2006 295(23):2727-41.)における乳癌発生率の低下と有害事象の発生状況に全国乳がん患者調査報告や人口動態統計のデータを組み合わせて、ホルモン療法剤予防内服による予後モデルを作成し、さらに東京都立駒込病院での乳癌患者医療費調査や国民医療費のデータを組み合わせて経済モデルを作成し、高リスクグループごとに余命延長と費用対効果を検討した。【結果】1)ゲイルモデルによる5年間2.01-3.0%の高リスク者が、35歳より5年間予防内服した場合、余命延長と増分費用効果比(ICER: Cost per YOLS)は、TAMで0.3日、3,341万円、RALで11.0日、403万円。50歳より内服した場合は、TAMで-1.0日、劣位、RALで7.7日、936万円。60歳より内服した場合は、TAMで-2.7日、劣位、RALで4.3日、2,309万円。2)同様に、3.01-5.0%の高リスク者が、35歳より内服した場合、余命延長とICERは、TAMで-2.9日、劣位、RALで7.8日、758万円。50歳より内服した場合は、TAMで-3.4日、劣位、RALで5.3日、1,519万円。60歳より内服した場合は、TAMで-4.2日、劣位、RALで2.8日、3,645万円。3)5.0%以上の高リスク者が、35歳より内服した場合、余命延長とICERは、TAMで32.8日、優位、RALで43.7日、優位。50歳より内服した場合は、TAMで22.8日、優位、RALで31.7日、優位。60歳より内服した場合は、TAMで12.5日、242万円、RALで19.7日、348万円であった。【考察】ゲイルモデルによる5.0%以上の高リスク者では、TAMおよびRALの両剤で予防内服が開始年齢にかかわらず費用対効果に優れており、両剤の比較では、効果はRALの方が優れているが費用対効果はTAMの方が優れていると示唆された。さらに、RALでは2.01-3.0%の高リスク者でも35歳より内服した場合に費用対効果に優れていることが示唆された。

SY-2-3

Signaling Proteome of Breast Cancer by High-Accuracy Mass Spectrometry

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Nano-scale liquid chromatography-tandem mass spectrometry (nanoLC–MS/MS) approaches, together with stable isotope labeling techniques, have been widely applied to quantitative proteomics. However, it remains elusive to analyze all tryptic phosphopeptides to complete phosphoproteome due to the abundant non-phosphopeptides that interfere with the MS detection of the phosphopeptides. Recently we developed highly specific enrichment method using hydroxy acid modified metal oxide chromatography (HAMMOC), where the chemo-affinity of metal oxides to phosphopeptides is enhanced by the addition of aliphatic hydroxy carboxylic acids.[1] The HAMMOC/nanoLC-MS/MS approach allows identify more than one thousand phosphorylation site from whole cell lysates. We applied it to breast cancer induced phosphorylation changes in cellular signal transduction network.

NanoLC-MS/MS analyses were conducted by using a ThermoFisher LTQ-Orbitrap mass spectrometer with a home-made nanoLC analytical column packed with C18 3 μm materials. Phosphorylation dynamics experiments were conducted as reported [2] except the treatment of MCF-7 cells by estradiol.

So far, we quantify 1994 non-redundant phosphorylation sites, 2873 phosphopeptides and 1033 phosphoproteins for estradiol-treated MCF-7 cells using HAMMOC/nanoLC-MSMS approach. We found 228 out of 1033 phosphoproteins have not been reported as proteins modified with phosphorylation. We also found the significant influence of estradiol treatment on the time-resolved phosphorylation of proteins belonging to EGFR signaling pathway.

[1]N. Sugiyama, T. Masuda, K. Shinoda, A. Nakamura, M. Tomita, Y. Ishihama, Mol Cell Proteomics 6 (2007) 1103.
[2]J.V. Olsen, B. Blagoev, F. Gnad, B. Macek, C. Kumar, P. Mortensen, M. Mann, Cell 127 (2006) 635.

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シグナル伝達とリン酸化プロテオミクス

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Signal Transduction and Phosphoproteomics

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タンパク質の翻訳後修飾は遺伝子解析では決して得られない情報であり、細胞の様々な機能をコントロールする必要不可欠な役割を果たしている。したがって、プロテオミクス研究において、翻訳後修飾情報を積極的に解析していくことは、細胞機能を理解する上できわめて重要である。様々な翻訳後修飾の中で、最も一般的な修飾の一つにタンパク質リン酸化があり、哺乳類では全タンパク質の 20-30%がリン酸化修飾を受けると言われている。特に、細胞内シグナル伝達ネットワークにおいては、タンパク質キナーゼがそのリン酸化により活性化され、その下流のタンパク質をさらにリン酸化するというシグナル伝達カスケードが存在し、細胞膜上の受容体に成長因子などの信号が入ってから、数 10 分～1 時間以内にこのリン酸化システムを通じて核内の転写因子等が活性化することが知られている。このシグナル伝達経路は経路毎に独立しているわけではなく、複数の経路が合流したり分岐したり複雑に入り組んでおり、また一つのタンパク質に対して複数のリン酸化サイトが存在し、それぞれが異なった経路を担っている場合も多い。したがって、細胞内シグナル伝達を理解するためには、個々の経路ではなくネットワーク全体の動きを調べる必要があり、またタンパク質単位ではなく、リン酸化サイト単位で解析する必要がある、さらに、受容体にシグナルが入った後の一定時間経過後のスナップショットではなく、ネットワーク全体の経時変化を解析する必要がある。

さて、チタニアやジルコニアなどの酸化金属はリン酸基に対して親和性を有することが知られている。最近、我々のグループでは、細胞全抽出物をトリプシンなどの消化酵素で切断して得られるペプチド混合物からリン酸化ペプチドを特異的に濃縮するために、酸化金属クロマトグラフィーの系を開発した[1]。通常、細胞全抽出物由来のペプチド混合物中のリン酸化ペプチド存在量は 1ppm 以下であり、酸化金属をそのまま担体として用いた場合、親和性はわずかだが大量に存在する非リン酸化ペプチド(主として酸性ペプチド)により、リン酸化ペプチドをほとんど検出することができなかった。そこで、酸化金属に対する親和性がリン酸基より弱く、カルボン酸よりも強いヒドロキシ酸で酸化金属を修飾することにより、細胞抽出物の消化ペプチド混合物からリン酸化ペプチドを選択的に濃縮する方法を確立し、HAMMOC 法(Hydroxy Acid-Modified Metal Oxide Chromatography)と名

づけた。乳酸修飾チタニアや 3-ヒロドキシプロピオン酸修飾ジルコニアを用いた HAMMOC 法とハイブリッド型イオントラップ-オービトラップ超高精度質量分析計を用いた nanoLC-MSMS 法を組み合わせることにより、100μg 程度の細胞抽出タンパク質試料から、1回の LC-MS 分析で 500-1000 個程度のリン酸化サイトを同定することが可能となった[2]。またチタニア-C18 の二相カラムを用いた全自動リン酸化ペプチド濃縮・分析システムも開発している[3]。

HAMMOC 法のシグナル伝達研究への応用として、癌細胞におけるリン酸化解析を行った。癌細胞においては一般的に増殖シグナルが著しく亢進しており、抗癌剤として各種キナーゼ阻害薬の上市も相次いでいるが、シグナル伝達ネットワーク全体に対する効果は不明な点も多い。我々は乳癌の増殖に関わる2つのシグナル伝達経路(ヒト上皮増殖因子受容体2(Her2)シグナリングおよびエストロゲン受容体(ER)シグナリング)に注目し、現在までに各種シグナルに対する 1000 個以上のリン酸化サイトの時間依存的リン酸化応答解析を行い、2つの経路の相互作用などを解析している。例えば、エストラジオール刺激により、Her2 シグナリングの下流タンパク質数種のリン酸化が確認されている。その他の応用例として、異物感染におけるシグナル伝達の変化をしらべるため、マラリア感染に注目した。熱帯熱マラリアは蚊を媒介し、ホストとなる哺乳動物の肝臓を経て赤血球に侵入し、そこで増殖を繰り返すことで、赤血球を破壊する。その一部は生殖母細胞に分化し、再び蚊を媒介として、生殖細胞となり、増殖する。そのユニークな生活環の中で、無性血液ステージに注目し、げっ歯類マラリア *Plasmodium berghei* のラット赤血球への侵入時におけるマラリアおよびラット赤血球側のシグナル伝達システムの経時的変化を解析した。赤血球においては、マラリアの侵入により著しくタンパク質リン酸化が亢進しており、その中でもある種のキナーゼファミリーの基質の選択的な増加が認められた。また、マラリア側においては、683 個のリン酸化サイトの時間依存的なリン酸化の変化が定量可能であり、侵入依存的にリン酸化が増加するサイト、減少するサイト、もしくは侵入に依存しないリン酸化サイトを同定することが可能であった。

本講演では HAMMOC 法を用いたリン酸化プロテオミクスによるシグナル伝達ネットワーク解析への応用を中心に紹介する。

文献

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PHOSPHOPROTEOME ANALYSIS FOR BREAST CANCER CELLS BY TWO-DIMENSIONAL NANOLC-MS USING A CALCINED TITANIA/C18 BIPHASIC COLUMN

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Introduction: Protein phosphorylation regulates many cellular signal transduction pathways that produce a range of biological responses in eukaryotes. Because these pathways are highly complex and crosstalk with each other, a systematic understanding of biological events requires novel technologies on specific enrichment of phosphoproteins/peptides for the comprehensive, time-resolved and phosphosite-specific mapping of *in vivo* phosphorylation.

Here, we will present an on-line automated system for phosphoproteome analysis using titania-based phosphopeptide enrichment followed by nanoLC-MSMS. Also, we will report the analysis of estradiol-induced signaling dynamics in breast cancer cells by modified titania chromatography.

Methods: Titania beads were prepared by calcination of commercial chromatographic titania beads at 800 °C to convert the crystalline form. 25 µg of the digested HeLa lysate proteins was loaded onto rutile-form titania/C18 biphasic column. After washing with 80% acetonitrile, the phosphopeptides were transferred from the titania phase to the C18 phase by injecting 0.5 % ammonium hydroxide, and a 60-min linear gradient LC-MS/MS analysis was subsequently carried out using a ThermoFisher LTQ-Orbitrap mass spectrometer.

Results and discussions: The obtained rutile-form titania exhibited higher selectivity in phosphopeptide enrichment than commercial titania when it was used in the absence of a competitive chelating reagent for non-phosphopeptides. For phosphoproteome analysis of HeLa cells, tryptic digests of the cell extracts were directly injected onto this on-line system, and 696 non-redundant phosphopeptides were successfully identified. In addition, the rutile-form titania exhibited different selectivity in phosphopeptide enrichment from commercial titania modified with lactic acid (LA), suggesting that rutile-form titania could be complementarily used with commercial titania to extend the phosphoproteome coverage. This on-line system as well as the off-line system with LA-modified commercial titania was applied to the analysis of breast cancer cells stimulated by estradiol. We found several pathways affected by estradiol including EGFR and insulin receptor signaling pathways.