

RESEARCH ARTICLE

Development of an immuno tandem mass spectrometry (iMALDI) assay for EGFR diagnosis

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The epidermal growth factor receptor (EGFR) is highly expressed in a variety of tumors, and is therefore an important biomarker for cancer diagnosis and a target for cancer therapy. We have developed a novel peptide-based immuno tandem mass spectrometry (iMALDI) diagnostic assay for highly sensitive, highly specific, and quantitative analysis of EGFR, which we have applied to the detection of the EGFR peptide in three cell lines and in a tumor biopsy sample. This assay is capable of detecting the EGFR target peptide bound to the antibody beads at attomole levels. The ability to directly obtain amino acid sequence data by MS/MS on any affinity-captured peptides provides specificity to this diagnostic technique. This avoids the problem of “false positives” which can result from the nonspecific binding that can occur with any affinity-based technique. The addition of stable-labeled versions of the target peptide (synthesized from stable-isotope coded amino acids) as internal standards allows absolute quantitation of the target protein.

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1 Introduction

Cancer is a complex disease with multiple mechanisms causing uncontrolled, abnormal cellular growth through oncogenic and anti-apoptotic pathways. One key enhancer of cell growth is the epidermal growth factor receptor (EGFR). EGFR, a member of the ErbB family of membrane-associated receptor tyrosine kinases, is a 170 kDa transmembrane glycoprotein consisting of an extracellular ligand-binding domain, a membrane-spanning domain, and an

intracellular cytoplasmic tyrosine kinase domain [1]. EGFR and its ErbB family members can homo- and heterodimerize in response to a number of ligands and elicit a variety of signaling transduction events that can affect cell growth and adhesion, migration, differentiation, and apoptosis [2]. EGFR up-regulation is frequently observed in a number of cancers, including those affecting the prostate, lung, ovary, and breast [3–6]. This up-regulation can deregulate multiple signaling pathways leading to increased proliferation and inhibition of apoptosis. High EGFR has been correlated with poor response to treatment and decreased survival times [5, 7]. Recently, many new EGFR targeted therapies have been developed that target either the extracellular ligand binding domain or the intracellular ATP binding domain [4, 8]. Con-

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Abbreviations: ABC, ammonium bicarbonate; EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; iMALDI, immuno tandem MALDI mass spectrometry

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sequently, the sensitivity, specificity, and quantitation capability of EGFR detection methods are of paramount importance.

Immunohistochemistry (IHC) is the most frequently used method to determine EGFR protein expression. However, IHC techniques are subject to a number of limitations with respect to the sensitivity and accurate quantitation of EGFR expression [1]. IHC also does not provide absolutely quantitative information concerning the expression and modification of multiple proteins in a single, high-throughput assay format. Other methods, such as Western blot analysis and ELISA, have many of the same limitations as IHC. Northern blotting or quantitative reverse transcription PCR (RT-PCR) is used to assess mRNA levels, but there may be problems with RNA degradation and contamination. Furthermore, mRNA levels do not always correlate with actual cellular protein levels or activity [9, 10], and they cannot predict post-translational modifications (PTMs).

As a result of these limitations, new proteomics technologies have been used to identify potential cancer biomarkers [9, 10], such as SELDI MS [11–13]. SELDI utilizes chemically modified surfaces to immobilize bait proteins (such as antibodies) and to capture native, intact analyte proteins for MS analysis. However, proteins are extremely sensitive to surface substrate physico-chemical properties and are easily denatured. MS analyses of full-length proteins suffer from limited resolution, which prevents identification based solely on mass. The heterogeneity, hydrophobicity, and limited solubility of proteins

may introduce significant loss-associated bias when attempting to detect proteins in complex biological samples.

We have developed a novel peptide-based immuno tandem mass spectrometry (iMALDI) diagnostic assay that circumvents many of the problems described above, and is distinguished by its ability to provide absolute specificity, absolute quantitation, and high sensitivity (Fig. 1). In our approach, anti-peptide antibodies are used for affinity capture. These antibodies are immobilized on inexpensive affinity beads rather than on the surface of a plate as is done in other protein chip technologies such as SELDI, thus eliminating the need for special surface chemistry.

The use of affinity beads also has the advantage of less-stringent sample storage requirements after antibody immobilization, and fewer complications resulting from denaturation of the capture antibody in solution. Peptides (which are more stable and less susceptible to denaturation than proteins) are detected and quantitated. In addition, due to their relatively small size (900–3200 Da) peptides are generally more soluble than proteins, especially in cases of hydrophobic and membrane proteins such as EGFR. Moreover, mass spectrometric analysis of peptides is rapid, accurate, sensitive, and specific because peptide molecular weights can be accurately determined by MS, while peptide sequencing can be done by tandem mass spectrometry (MS^2). This allows the analyst to distinguish nonspecifically bound, and even cross-affinity-bound peptides from the target peptide.

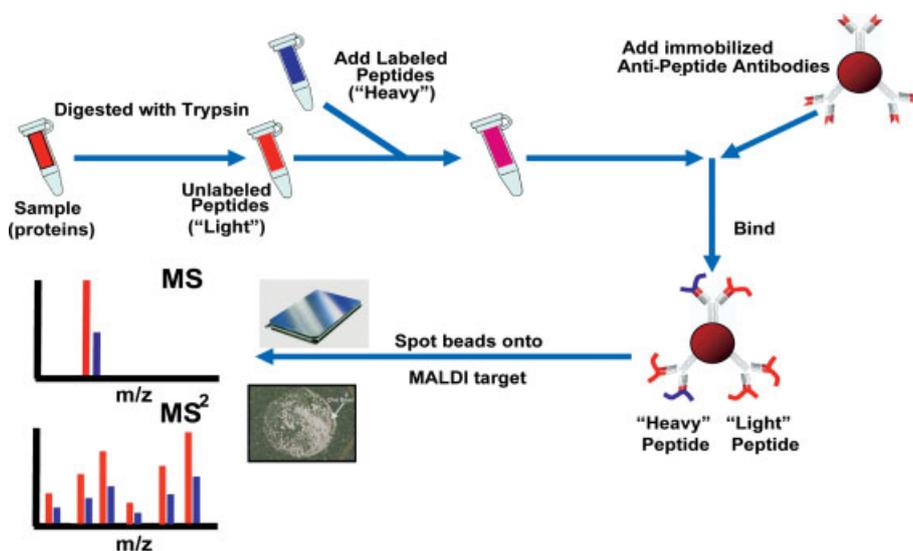


Figure 1. Analytical scheme of the iMALDI assay. Epitope-containing peptides, synthesized using isotopically labeled amino acids, are added to a proteolytic digest and are subsequently incubated with the appropriate antibody beads to immunoprecipitate the epitope-containing peptides of interest. After immunoprecipitation of the differentially labeled peptides, the antibody beads are arranged in a microarray/spot format on the MALDI-target plate. Adding MALDI matrix solution to the affinity-bound peptides elutes the peptides from the immobilized antibodies, permitting MALDI analysis of the peptides. The relative abundances of the molecular ion signals corresponding to light and heavy peptides (isotopically labeled, synthetic epitope peptides) are used to quantify the amount of this protein in the original sample. Absolute specificity can be achieved by mass spectrometric sequencing of the epitope containing peptide, using MALDI-MS/MS.

Either relative or absolute quantitation is possible, depending on whether the target peptides from two samples have been differentially labeled (relative quantitation), or if a stable-isotope labeled peptide has been added as an internal standard (absolute quantitation). In addition, because extensive protein separation is not required prior to analysis, and because low-abundance proteins can be analyzed in the presence of high-abundance proteins, our iMALDI approach provides both speed and dynamic range.

In this manuscript, we describe an iMALDI peptide assay for detection of EGFR, based on affinity capture of the EGFR peptide possessing the amino acid residues 963–975. This iMALDI assay for the EGFR peptide is capable of fast, sensitive, specific, and quantitative detection of EGFR in mammalian breast cancer cell lines and tumors.

2 Materials and methods

2.1 EGFR protein

EGFR (human) protein was purchased from Sigma (Saint Louis, MO).

2.2 Breast cancer cell lines and cell lysates

ME16C, SUM102 (<http://www.asterand.com/Asterand/BIOREPOSITORY/102PT.aspx>), and MCF-7 cell lines were obtained from the Perou lab (LCCC, UNC-CH) in the form of cell lysates. Whole cell protein lysates were extracted from the cell lines using Pierce's cell line (M-PER) protein lysates extraction kit. The protein content of the whole cell lysate was determined using Pierce's Micro BCA assay according to manufacturer's protocol.

2.3 Human Breast Cancer tumor

Tumor lysates were obtained from the Perou lab (LCCC, UNC-CH) in the form of cell lysates. Tumor lysates were prepared from the tumor tissue using Pierce's Tissue (T-PER) lysate extraction kit (Pierce). This tumor sample was procured from the UNC-CH Tissue Procurement Facility using an IRB-approved protocol.

2.4 Tryptic digestion

Proteolytic digestion was carried out in 25 mM ammonium bicarbonate (ABC) (Sigma) at 37°C overnight. The enzyme/substrate ratio of trypsin (Sequencing-grade modified, Promega) to protein was approximately 1:10.

2.5 Antibody production and immobilization of antibodies on beads

An antibody was raised against the EGFR tryptic peptide $^{963}\text{MHLPSPTDSNFYR}^{975}$ by Cocalico Biologicals (Reams-

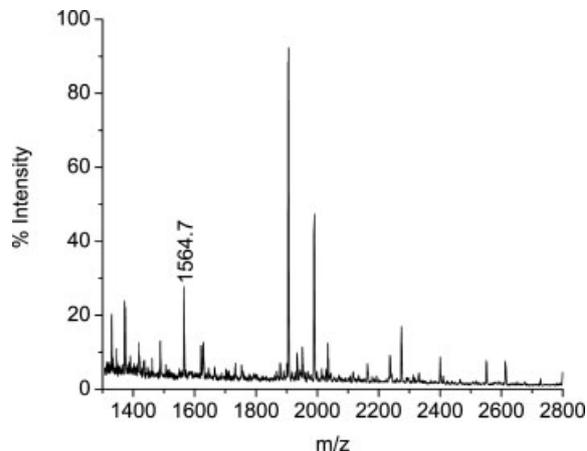


Figure 2. Selection of EGFR peptides to be used for raising antibodies for the EGFR iMALDI. MALDI-MS of proteolytic EGFR peptides, obtained by in-solution digestion of EGFR with trypsin. The “true” (C-terminal cleavage of K or R residues) tryptic peptide of EGFR of m/z 1564.7 (sequence shown in inset) was selected for antibody production based on its high sensitivity in the MALDI-MS mode.

town, PA). This peptide showed high sensitivity in the MALDI-MS mode (Fig. 2). This antipeptide antibody was then covalently immobilized on CNBr-activated Sepharose (Amersham Pharmacia) according to the manufacturer's instructions, and as described in detail in our publication [14]. Briefly, the Sepharose beads react with primary amine groups on the antibody, thereby covalently linking the antibodies to the beads. Excess binding sites on the beads are blocked by incubation with an amine-containing buffer, (here, Tris buffer), and the process is completed by a series of washes at alternating acidic and basic pH.

2.6 Immunoprecipitation protocol

Affinity binding was carried out in compact reaction columns (USB) after loading with an aliquot of antibody beads (1–5 μL). The beads were washed four to five times with 400 μL of 0.1 \times PBS. The synthetic EGFR peptide ($\text{C}^{963}\text{MHLPSPTDSNFYR}^{975}$) (Biosynthesis) has an additional N-terminal cysteine residue which was added for ready conjugation with carrier proteins. The synthetic peptide was dissolved in water/buffer, and then diluted to a series of concentrations, e.g., 150, 15, 1.5 $\text{ng}/\mu\text{L}$, etc. A 10 μL aliquot of each solution was incubated separately with a small amount (1–5 μL) of antibody beads to immunoprecipitate the epitope-containing peptide. Immunoprecipitation of tryptic digests of the EGFR protein, cell lysates, or tumor biopsy samples were carried out similarly. PBS was incubated with the antibody beads or unconjugated agarose beads as negative controls.

After incubation for 2–4 h at room temperature with end-to-end rotation on a “Labquake” shaker (Lab Industries), the beads were washed six times with 400 μL of freshly prepared

50 mM ABC, or (in cases where high levels of nonspecific binding was expected), three times with 400 mM of NaCl (Sigma) followed by three times with 50 mM ABC. The beads were resuspended in a small volume of 50 mM ABC (1–5 μ L) and an aliquot of the beads (0.5 μ L) was spotted directly onto the MALDI target as described in Section 3.

2.7 Isotopic labeling for absolute quantitation

The EGFR aa 963–975 peptide ⁹⁶³MHLPSPDTSNFYR⁹⁷⁵ containing an isotopically labeled leucine at position 965 (underscored) (heavy) was synthesized at the UNC peptide synthesis facility, using a ²H-labeled Fmoc leucine purchased from C/D/N Isotopes (Quebec, Canada). The increase in mass from the unlabeled EGFR aa 963–975 peptide was 10 Da. The synthesis was performed according to the Fmoc strategy and is described in detail elsewhere [15].

2.8 Absolute quantitation

The isotopic-labeled (heavy) peptide was used as an internal standard. The heavy peptide was weighed on a microbalance and dissolved in water (HPLC grade, Pierce). The exact concentration of the heavy peptide solution was determined by amino acid analysis at the UNC Center for Environmental Health and Susceptibility. Peptides and amino acid standards were dried, hydrolyzed under HCl vapor for 1 h at 150°C, and analyzed by LC-MS/MS. Quantitation was performed by the analysis of serine and proline transitions using norleucine as an internal standard.

Various amounts of the heavy peptide was spiked into a SUM102-cell tryptic digest, immunoprecipitated on anti-EGFR peptide antibody beads, and quantitated by direct MALDI-MS analysis of the beads on the MALDI target.

2.9 Mass spectrometric analysis

All MALDI-MS and MS/MS experiments were performed on a MALDI-TOF instrument (Ultraflex I) from Bruker Daltonics (Billerica, MA) using the Bruker Anchor-chip™ MALDI-target plates (400 or 600 μ m/384 spot format). The matrix used for all experiments was CHCA (Sigma) after recrystallization from hot methanol. The solvent for CHCA was ACN (Caledon Laboratories)/ water (HPLC grade, Pierce)/ TFA (Pierce) (50:49.9:0.1 by volume).

For placement of beads on the MALDI target, 50 mM ABC or 0.1 \times PBS solution was added to resuspend the antibody beads after immunoprecipitation. Following placement of the antibody beads on the target, 0.5 μ L of a saturated solution of CHCA matrix was added to the beads and the spot was allowed to dry at room temperature.

3 Results and discussion

In previous studies on model peptides, we have shown that our peptide-based iMALDI technique is capable of detecting and quantifying attomole-to-low femtomole levels of peptides bound to antibody beads, as well as specifically detecting target peptides affinity-purified from complex matrices such as cell lysates. MS/MS data suitable for protein identification *via* database searching or *de novo* sequencing can be obtained from these affinity-bound peptides. We have also shown that this technique is compatible with a microarray format for high-throughput analysis [16]. This technology is capable of determining levels of proteins such as p53, prostate-specific antigen (PSA), and *Francisella tularensis* IglC, using commercially available or customized antipeptide antibodies [14, 17]. In the current study, we have used this technique for the detection and quantitation of EGFR protein using a customized antibody which had been raised against an EGFR peptide, selected because of its high sensitivity in MALDI-MS (Fig. 2).

3.1 Sensitivity studies – synthetic peptide

Using our iMALDI technology, the detection limit for the synthetic EGFR peptide was determined to be in the low attomole range in buffer (1 attomole). In contrast, with MALDI-MS analysis of the peptide solution without enrichment through iMALDI, the detection sensitivity was in the low femtomole range in buffer (5 fmol) (Fig. 3). This loss of detection sensitivity is likely because during the sample preparation process, the peptides in solution are adsorbed by the walls of the sample tubes and by the pipette tips. In contrast, using the enrichment procedure, the peptides are bound to the antibody beads. This greatly reduces the peptide loss due to adsorption.

There is an increase in S/N ratio for the signal from the 1 amol with iMALDI (Fig. 3b) compared to the 10 fmol and 100 amol levels. The number of beads placed on the target is used for calculating the amount of target peptide spotted. The co-crystallization is not uniform, and for the 1 amol level, apparently a better crystal was sampled than for the 10 fmol and 100 amol levels. Therefore, the signal from the “hot spot” of the former has an increased S/N ratio compared to the signals from the latter two. However, if labeled internal standards are used, this will not affect quantitation in a sample.

3.2 Breast cancer cell lines

SUM102 and ME16C are both EGFR-expressing mammalian breast cancer cell lines. SUM102 is a tumor-derived basal-like cell line [18]. ME16C is a human mammary epithelial cell line immortalized with hTERT with characteristics of the basal-like subtype [18].

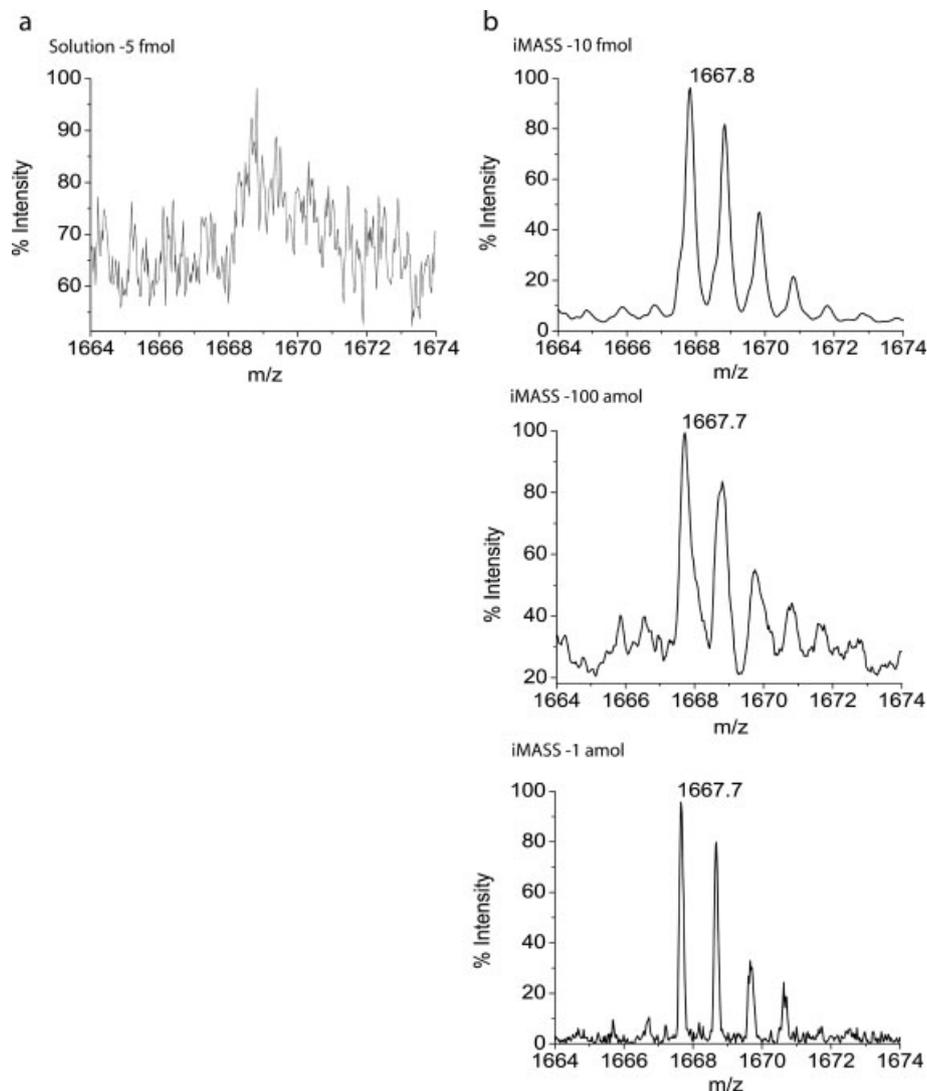


Figure 3. Comparative study to determine the sensitivity of MALDI-MS analysis of the EGFR peptide aa 963–975 (a) from solution and (b) using our iMALDI technology. The EGFR peptide aa 963–975 of $m/z = 1667.7$ was used for raising antibodies, and is identical to the native EGFR peptide aa 963–975, with the addition of an N-terminal cysteine residue (C⁹⁶³MHLPSPTDSNFYR⁹⁷⁵) to facilitate conjugation with carrier proteins.

3.2.1 SUM102 cell lysate

EGFR was determined in a SUM102 cell lysate equivalent to ten cells. The cell lysate was digested and incubated with a small aliquot (1–5 μL) of anti-aa 963–975 EGFR antibody beads. One-tenth of the antibody beads were spotted on the MALDI target plate and analyzed directly. The singly charged, epitope-containing, tryptic peptide (aa 963–975 MHLPSPTDSNFYR) from the EGFR protein was observed at $m/z = 1564.7$ (Fig. 4).

Absolute quantitation of EGFR in SUM102 was also performed. By “absolute quantitation,” we mean that, unlike “relative quantitation,” where the ratio of the peptide concentrations in two samples is compared, what is determined is the absolute amount of the peptide relative to a standard of known concentration. For absolute quantitation of peptides, synthetic stable-isotopically labeled peptides (heavy peptides) are used as internal standards. These heavy pep-

tides are identical in amino acid sequence to the native epitope-containing peptides (light peptides) of the protein in the sample, but are higher in mass and thus distinguishable by MS. The heavy peptide is added to the proteolytically digested protein sample prior to incubation with the immobilized antipeptide antibody. Following affinity enrichment of the target peptides, the MALDI-MS analysis of the antibody beads shows doublets of ion signals from the light and heavy peptides, whose intensity ratios can be used for absolute quantitation.

For quantifying the EGFR aa 963–975 peptide ⁹⁶³MHLPSPTDSNFYR⁹⁷⁵, we had the heavy peptide (H) synthesized with an isotopically labeled leucine at position 965. This peptide (heavy peptide, H) is identical in amino acid sequence to the native epitope-containing peptide (light peptide, L, $m/z = 1564.7$) for the anti-EGFR aa 963–975 antibody, but is 10 Da higher in mass ($m/z = 1574.7$) due to replacement of ten ¹Hs with ten ²Hs. In these

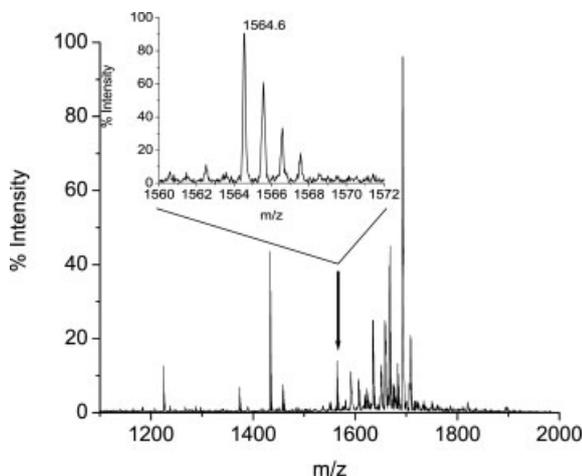


Figure 4. Detection of EGFR using the EGFR iMALDI in mammalian breast cancer cells. MALDI-MS spectrum of peptides affinity-bound to anti-aa 963–975 EGFR antibody beads obtained after lysis of SUM102 cells, followed by proteolysis with trypsin. The singly charged, epitope-containing, tryptic peptide aa 963–975 MHLPSPTDSNFYR of the EGFR protein is observed at $m/z = 1564.7$ (see inset).

experiments, heavy aa 963–975 peptide (H) was added in various amounts to the proteolytic digest of SUM102 cell lysate, and was immunoprecipitated using the anti-EGFR aa 963–975 antibody, immobilized on affinity beads (Figs. 5a and b).

Analysis of an aliquot of these beads (*ca.* 10%) spotted directly on the MALDI target demonstrates that the mono-

isotopic ion abundances of the two peptides can be distinguished from one another by a mass difference of 10 Da. The logarithmic plot (Fig. 5c) shows a linear correlation (slope is approximately one) between the ratio of the two peptides (H/L) and the amount of H spiked in as internal standard over near two orders of magnitude. This demonstrates the accuracy of this technique for the absolute quantitation of EGFR peptides using an internal standard.

Using this standard curve, we determined the amount of EGFR in SUM102 cells in which EGFR is highly up-regulated. The molar amount of EGFR peptide is equal to the amount of digested EGFR protein in the cell lysate. This amount of digested EGFR protein is the same as the amount in the original cell lysate and cells, assuming 100% efficiency of the digestion reaction and the protein extraction upon cell lysis [19]. The concentration of EGFR was determined to be 0.65 amol/cell, which is approximately 390 000 EGFR molecules *per* SUM102 cell. This result is consistent with the EGFR number ($\sim 50\,000$) in HeLa cell in the literature [20].

Our iMALDI technology can provide the highest possible specificity by determining two molecular characteristics of the epitope-containing peptides: (i) the molecular weight, typically measured by MALDI-MS within an error of 100 ppm or better and (ii) the amino acid sequence, by performing MS/MS using MALDI-MS/MS.

3.2.2 ME16C cell lysate

The iMALDI assay was used to accurately detect immuno-affinity-enriched EGFR aa 963–975 peptide obtained from the ME16C cell lysate. (Data not shown)

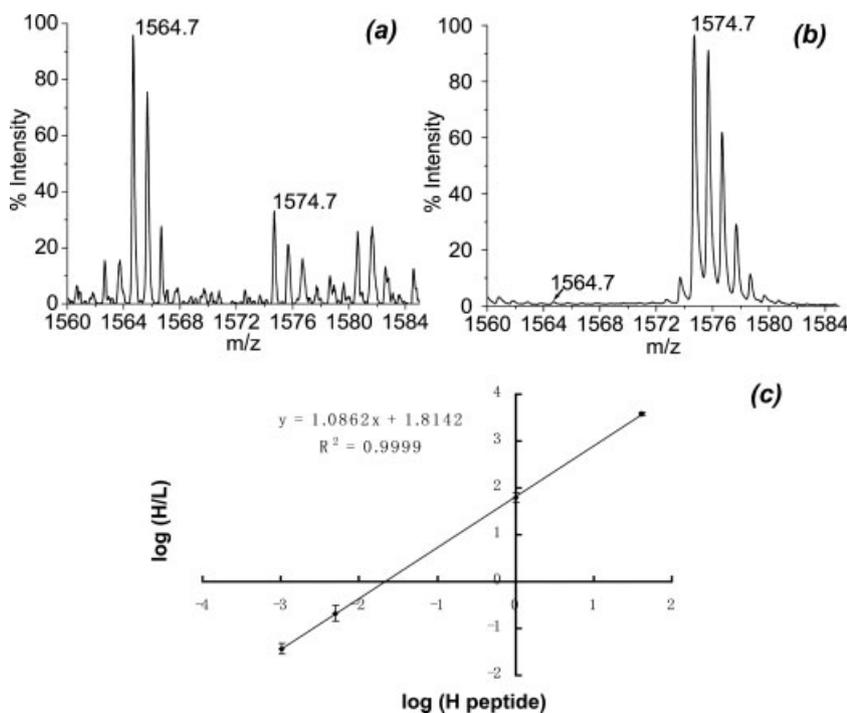


Figure 5. Quantitation of EGFR in SUM102 cells using the EGFR iMALDI. Absolute quantitation of the EGFR peptide MHLPSPTDSNFYR (aa 963–975) (L, light peptide, $m/z = 1564.7$) in SUM102 cells. Cells were lysed, digested, and incubated with known amounts of heavy peptides (H, $m/z = 1574.7$) as internal standards: (a) 0.1 pmol, (b) 1 pmol of H. (c) Logarithmic plot of the observed ratios of monoisotopic ion abundances of H and L in the MALDI-MS spectra *versus* the absolute amount of H added.

3.2.3 MCF-7 cell line

The assay was repeated for lysates from the MCF-7 cell line, which is a tumor-derived luminal cell line that does not express EGFR. The target peptide was not detected in these samples.

3.3 Human breast cancer tumor biopsy sample

We analyzed an estrogen receptor negative and HER2-negative breast tumor biopsy sample, BR97-0137B, which had been previously shown through gene expression analysis to be a grade III infiltrating ductal carcinoma that has gene expression characteristics of the basal-like subtype and high gene expression of EGFR [2].

The iMALDI assay revealed ion signals from the target peptide ($m/z = 1564.7$) from the digest of BR97-0137B tumor tissue lysate with an $S/N > 5$ (Fig. 6). It is important to note that for the iMALDI analysis less than 1/25 of the biopsy sample has been used. This demonstrates the applicability of this assay to clinical samples such as tumor biopsies.

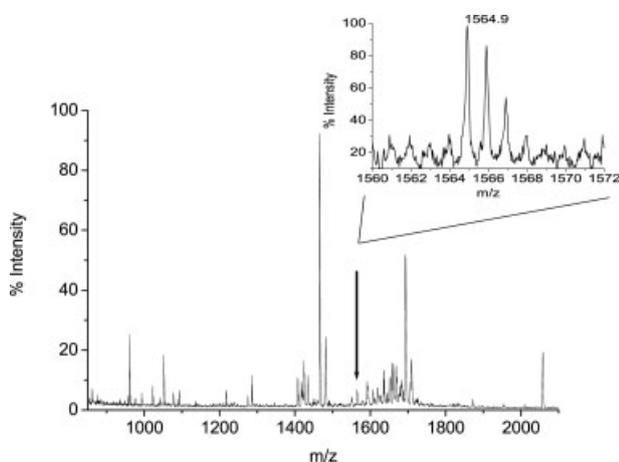


Figure 6. Detection of EGFR using the EGFR iMALDI in the basal-like primary human tumor BR97-0137B. MALDI-MS spectrum of peptides affinity-bound to anti-aa 963–975 EGFR antibody beads obtained after lysis of BR97-0137B cells, followed by tryptic proteolysis. The singly charged, epitope-containing, tryptic peptide aa 963–975 MHLPSPTDSNFYR of the EGFR protein can be observed at $m/z = 1564.7$ (see inset).

3.4 Specificity

When comparing the iMALDI mass spectra from the peptide mixtures derived from the SUM102 cells (Fig. 4) and BR97-0137B tumor tissue lysate (Fig. 6), we found a similarity in the detected ions in the mass range m/z 1500–1700. These ions are most likely peptides from the digest of cell lysates, nonspecifically bound to the antibody beads. More washes may reduce the amount of these nonspecifically

bound peptides, but even in the presence of these non-specifically-bound peptides, the affinity-bound target peptide is still detectable by MS. These nonspecific binders to the antibody would probably cause “false positives” in an ELISA-type assay, which does not have the capability to distinguish between bound analytes on the basis of their molecular weights. This clearly shows the advantages of the high specificity of the iMALDI technique.

The accuracy of the molecular weight of the EGFR peptide was within 75 ppm or better (Fig. 4). In addition, we have shown that this technique is capable of obtaining sequence information on the affinity-bound EGFR aa 963–975 peptide from the digest of ME16C cell lysate by MALDI-MS/MS (Fig. 7). MS/MS analysis provides sequence-specific ions, such as y_1 , y_5 , and y_{12} which are highly abundant, and several others with slightly lower abundances. Even though a few sequence-specific ions, such as y_7 and y_9 , are absent, complete sequence coverage is not necessary for confident assignment. Moreover, considering the fact that y_7 and y_9 are produced by C-terminal fragmentation of Pro, their absence actually increases confidence in the assignment. In the existing NCBI database, searching with MS data resulted in 33 129 hits out of 2 million entries, while combining MS and MS/MS data resulted in just one hit. Sequence blast of this peptide was also performed on the human genome. The most significant hits are all related to EGFR, besides EGFR, either its isoforms, or one of its domains, mainly the tyrosine kinase domain. This clearly demonstrates that the combination of these two mass spectrometric approaches (MALDI-MS and MALDI-MS/MS), inherent in our iMALDI assay, is capable of unambiguous identification of affinity-bound peptides, and therefore, permits highly specific detection of EGFR.

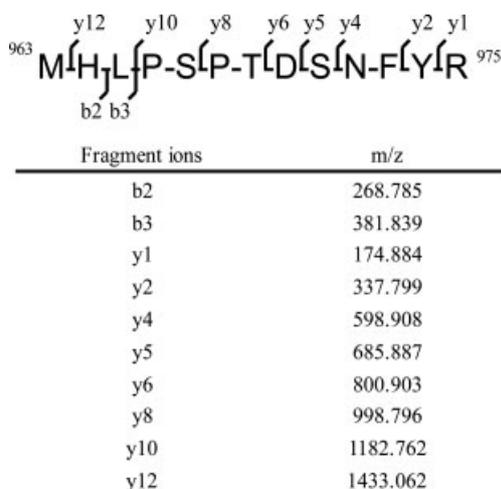


Figure 7. Highly specific detection of EGFR in ME16C cells by mass spectrometric sequencing of the immunoaffinity enriched EGFR aa 963–975 peptide using the EGFR iMALDI. The sequence-specific b and y -ions of the peptide at $m/z = 1564.7$ affinity-bound to anti-aa 963–975 EGFR antibody beads observed in MALDI-MS/MS spectrum after lysis and proteolysis of ME16C cells.

Since this technique is based on affinity capture by the antibody, there is the possibility of false negatives due to target sequence variation. If there had been a substitution of a key amino acid residue in the epitope-containing region, the peptide may not be captured by the antibody, resulting in a “false negative.” This iMALDI for EGFR has been based on a single peptide because this peptide (aa 963–975 MHLPSPTDSNFYR of EGFR) is highly sensitive in MALDI-MS (Fig. 2), and among all antibodies generated against the EGFR tryptic peptides with high sensitivities in MALDI-MS, the anti-aa 963–975 MHLPSPTDSNFYR has the highest affinity. To eliminate the problem of “false negatives,” other antibodies can be added to the assay to target multiple epitopes providing more coverage of the protein. Moreover, since polyclonal antibodies are used in this assay, more than a single epitope may be contained within a particular stretch of amino acids. Therefore, even modified peptides are likely to contain enough of an unmodified stretch to be captured by these polyclonal antibodies. It is highly unlikely that all target peptides would be mutated, so several characteristic peptides should still be detected by this assay. Mutations in the DNA could lead to failure of the PCR-based assays and unexpected amino acids in the target peptide sequences. These would be detected by the MS/MS sequencing of the captured peptides, and the homology would be revealed. In addition to *de novo* peptide sequencing methods, current database searching programs, such as the MASCOT™ software, can identify peptides with substitutions and/or deletions. All these will decrease the possibility of “false negatives.”

4 Concluding remarks

Using our iMALDI method, using the anti-EGFR aa 963–975 antibody, we have detected EGFR in mammalian breast cancer cell lines ME16C and SUM102 where it is known to be present, and not in the MCF-7 cell line where it is known NOT to be present. Using this technique, we were also able to detect EGFR in a human breast tumor biopsy sample. This iMALDI assay for EGFR is capable of achieving sensitivities in the low attomole range, with high specificity, and is also capable of absolute quantitation of target peptides with a linear dynamic range of over two orders of magnitude. The sensitivity of the iMALDI assay for EGFR allows the detection of this protein from the cell lysate corresponding to a few (≤ 10) SUM102 cells, while the specificity of the assay ensures a low rate of false positives.

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