いて機能解析を行い、新規大腸癌治療薬のターゲット候補因子 FAM83H と CK-1αを見出した。

大規模リン酸化タンパク質の探索から検証までのワークフローを確立した。

また、アルツハイマー病のサロゲートマーカーAPL1βの超高感度定量の実現に成功した。

E-2. 疾患関連蛋白質の解析基盤の研究：角田慎一

創薬を指向した疾患プロテオミクス研究を推進するため、この 5 年間で、1) 抗体プロテオミクス技術、2)In vivo biotinylation 法、3) Exosome に着目した解析法を確立した。

抗体プロテオミクス技術を活用し、これまでに乳がんとの関連が知られていない新規の乳がん特異的蛋白質を多数同定し、その中で EphA10 が、乳がんのリンパ節転移やステージの進行に伴って高率に発現すること、Her2 陰性症例や、TNBC 症例にも発現している一方、正常組織では精巣にのみ発現が認められ、これまで治療法のない症例の有用な創薬ターゲットになりうることを示した。また、肺がん特異的かつリンパ節転移に相関する OSBPL5、CALU を同定した。

In vivo biotinylation 法によって、リンパ腫血管に高発現する BST-2 を同定し、これに対するモノクローナル抗体が抗腫瘍効果を示した。

Exosome に着目したプロテオーム解析により、新規肺がんマーカー候補として、EphA2 と CNTN1 を同定した。以上の成果は、疾患プロテオミクス研究を推進するための有用な基盤技術になりうることを示しており、プロテオーム創薬に貢献することが期待される。

E-3. プロテオミクス手法による癌の創薬標的分子探索：仲　哲治

本研究の結果、卵巣明細胞腺癌の抗癌剤耐性分子として Annexin A4 を同定した。Annexin A4 の強制発現は漿液性腺癌細胞株 OVSAHO に対してカルボプラチンの耐性を誘導したが、その機序として Annexin A4 がカルボプラチンの細胞内取り込みを抑制し、細胞外への排出を促進することが明らかになった。以上の結果から、Annexin A4 は抗癌剤耐性を克服するための標的分子となり得ると考えられる。

細胞表面膜タンパク質のビオチン標識による濃縮と iTRAQ 法による網羅的な定量解析は正常細胞と比較して癌細胞に高発現する癌抗原の同定に効果的であることが示された。本手法により BST2 は子宮内膜癌の治療標的としての有用性が *in vitro, in vivo* の実験結果より示された。本手法は様々な癌種に対して応用出来るため、優れた創薬基盤技術であると考えられる。

悪性黒色腫と正常皮膚組織の手術検体を用いて iTRAQ 法による網羅的なタンパク質発現定量解析を行った結果、悪性黒色腫に高発現するタンパク質として Periostin を同定した。Periostin は悪性黒色腫の増殖に関与することが *in vitro, in vivo* の実験結果より示された。悪性黒色腫に対して Periostin が創薬標的分子となり得る可能性が示唆された。

E-4. ターゲットプロテオミクスを用いた網羅的タンパク質解析技術の開発とバイオマーカー探索への応用：中山敬一

より信頼性の高い MRM method ライブラリーの構築によって容易に多検体での MRM 解析（iMRM 法）が可能となり、iMRM 法による精密なタンパク質発現絶対量の計測が実施できた。また、SWATH 法を導入することでより網羅的な絶対定量法の構築の目処がたった。さらに SWATH 法のリン酸化定量解析への応用の可能性も示すことができた（iSWATH 法）。これらの方法を組み合わせて利用することで容易にパスウェイワイド・システムワイドなタンパク質の絶対量計測やリン酸化変動計測が可能であり、バイオマーカー探索実施のための大規模データ取得において次世代の有効な手段となることが十分に期待される。

E-5. 創薬バイオマーカー探索研究基盤の確

立とその活用：平野　久

本研究において、卵巣明細胞腺癌を研究対象として、疾患関連タンパク質の検出・同定、創薬標的分子あるいは診断マーカーとしての有用性の検証、診断マーカーのアッセイに関する効率的な方法がわかった。最も効率的と考えられる方法を用いて、実際に卵巣明細胞腺癌で発現が変動するタンパク質を検出し、創薬標的分子として利用可能なタンパク質を見いだすことができた。また、診断マーカーとして実用化できる可能性が高いタンパク質を発見することができた。さらに、質量分析装置を用いて診断マーカーをアッセイできる可能性が生まれた。

E-6. 2DICAL による微量たんぱく質解析技術の研究：尾野雅哉

2DICALを用いた血漿バイオマーカーの探索、および、多数検体での検証に成功し、本事業での研究成果を上げることができた。今後、バイオマーカーとして実用化するためには、臨床の現場で測定を行い、その有用性につての最終的判断が下せるデータを獲得していかなければならない。また、肝臓癌の診断治療に有用なバイオマーカーの開発を行うことにより、組織検体を用いたバイオマーカー開発とともに、リン酸化ペプチドを利用した新規の診断治療にかかわるバイオマーカーの開発が重要な研究課題となっていくことが明らかになった。本研究期間に2DICALのバージョンアップも行なわれ、今後、微量タンパク質の解析がさらに進み、より有用なバイオマーカーが開発されることが期待される。

E-7. 循環器疾患に関連する微量たんぱく質解析技術の研究：寒川賢治、南野直人

心血管系培養細胞を用いたバイオマーカー探索の結果、非心筋細胞培養では細胞外マトリックスに加え、生理活性ペプチド前駆体、サイトカイン等の断片ペプチドが多数観測された。

循環器疾患におけるバイオマーカー探索対象として心不全を選定し、イヌ心不全モデル、マウス心筋症モデルについて定量プロテオーム解析を実施した。以前より報告されている収縮機能関連タンパク質、細胞内カルシウム制御系タンパク質以外に、エネルギー代謝系酵素群の顕著な減少が認められ、疾患の成因や増悪機構を探る上で重要と考えられた。

E-8. 精神・神経疾患に関連する微量タンパク質解析技術の研究：高坂新一

精神・神経疾患のプロテオーム解析を行うにあたって、髄液の有用性を最大限活用できるように、微量タンパク測定系、特に前処理法を確立した。これによって、質量分析計QSTARによる解析で常に300種近くの蛋白質が同定できる系を確立できた。分析装置の発展があれば、さらに多くのタンパク質の同定が可能になるであろう。

また、髄液種集の体制整備、採取後の処理法、食事の影響など、今後髄液を用いた診断や研究を行う際に不可欠な条件検討を行えたことは、今後の研究に大いに資するものと考えられる。

E-9. 新規糖鎖腫瘍マーカーおよび血液中腫瘍由来DNAの研究：加藤菊也、宮本泰豪

ST1Hを認識する単クローン抗体の作成方法を再検討する必要がある。Neu5AcやKDN付加されたN型の遊離糖鎖の検出方法を確立し、マーカーとしての有用性の検討が必要である。

血漿中腫瘍由来DNA解析は前向き検証試験での検証段階に到達している。次世代シーケンサーを用いた方法は汎用性があるため、他のがん遺伝子がん抑制遺伝子についても応用可能と考えられる。

E-10. 血清・血漿の前処理法に関する微量タンパク質解析技術の研究、血清・血漿を用いたプロテオーム解析の臨床検査応用：野村文夫

血清プロテオーム解析によるバイオマーカー探索とそのバリデーションのために必要な検体採取のプロトコールを作成し、ペプチドーム解析のための検体前処理法を開発した。これらの方法を用いて慢性血栓塞栓性肺高血圧症、アルコール性臓器障害マーカーの探索

をおこなうと同時に、歯肉溝浸出液を用いた歯周病マーカーの探索も行った。一方、早期がんの診断においては発現プロテオミクスのみの解析には限界があるので、血清自己抗体からのアプローチも行い、原発性肝細胞癌の早期診断に有望な血清自己抗体の検出に成功した。

E-11.：脳腫瘍に関連する微量タンパク質解析技術の研究：統合プロテオミクスによるバイオマーカー／治療ターゲットとなる脳神経系腫瘍組織細胞内シグナル分子群の解析：荒木令江

本研究では、1) 悪性グリオーマの抗がん剤感受性に関わる分子シグナルとして、Vimentin とその翻訳後修飾に関わる責任酵素群、およびその活性化シグナル分子群による新規の Tyrosine Kinase receptor-Vimentin activation loop が悪性グリオーマの治療抵抗性に大きく関わっていることを明らかにした。2) グリオーマ幹細胞(GIC)の分化に、インテグリンαV と FN の発現と相互作用が重要であること、これらの阻害剤が GIC の分化抑制に有用であり抗がん剤との併用で GIC の薬剤耐性を感受性に転ずることが可能であることを示し、GIC は自らインテグリンと ECM の発現を介して分化を制御する微小環境“分化ニッチ”を形成することを提唱した。3) NF1 の病態解析から、NF1-RAS-MAPK/ PI3K-Protein T-mTOR 活性化シグナルおよび Dynein IC2-GR-COX-1 の一連の特異的活性化シグナルネットワークが検出され、Protein T や COX-1 が NF1 の悪性化マーカーおよび治療ターゲットとしての可能性が示唆された。

E-12. 自己抗体を活用した難治性がんのバイオマーカー探索研究：中村和行

C 型肝炎ウィルス感染に起因すると考えられる肝細胞がん（HCV-HCC）の組織特異タンパク質バイオマーカーの探索技術の改良を行い、従来の HSP70, MnSOD, peroxiredoxin に加えて糖鎖化 ApoE および ATP 合成酵素アイソフォームなどを新たなバイオマーカー候補蛋白として同定した。

一方、患者血清中の自己抗体を活用した PROTEOMEX 法とプロテインチップ技術の改良を行い、HSP70 の C 末端領域が HCV-HCC の特異バイオマーカー候補として有望であることを明らかにした。さらに、自己抗体を用いた PROTEOMEX 法により乳がんのバイオマーカー候補として抗 cyclophilin A 抗体を同定した。

F. 研究発表

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