Ectokinases as novel cancer markers and drug targets in cancer therapy

Garif Yalak¹,² & Viola Vogel²

¹Harvard Medical School/Harvard School of Dental Medicine, Department of Developmental Biology, Harvard University, Boston, Massachusetts 02115
²Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, ETH Zurich, Switzerland

Abstract

While small-molecule kinase inhibitors became the most prominent anticancer drugs, novel combinatorial strategies need to be developed as the fight against cancer is not yet won. We review emerging literature showing that the release of several ectokinases is significantly upregulated in body fluids from cancer patients and that they leave behind their unique signatures on extracellular matrix (ECM) proteins. Our analysis of proteomic data reveals that fibronectin is heavily phosphorylated in cancer tissues particularly within its growth factor binding sites and on domains that regulate fibrillogenesis. We are thus making the case that cancer is not only a disease of cells but also of the ECM. Targeting extracellular kinases or the extracellular signatures they leave behind might thus create novel opportunities in cancer diagnosis as well as new avenues to interfere with cancer progression and malignancy.

Introduction

Since the fight against cancer is far from being won, there is a need to think of new strategies to identify alternative targets for cancer diagnosis and combinatorial therapies. Current challenges include the desire to detect cancer much earlier, to prevent or reduce the emergence of acquired drug resistance [1], and to reduce the often
lethal side effects. Even more challenging is the fact that different cancer cells from the same tumor can use different pathways to achieve drug resistance [2]. The complexity of pathways that can lead to drug resistance prevents to predict which treatment modality might finally allow the host rather the cancer to survive [3, 4]. Continued chemotherapy will target only a subset of cancer cells, while the resistant cells continue to grow [2]. New strategies are therefore needed to target nonresistant and resistant cancer cells. Protein phosphorylation is the key regulatory posttranslational modification exploited for intracellular signaling [5–7], and kinases require sufficiently high ATP levels to transfer a phosphate group. Today, it is believed that one third of human proteins are phosphorylated [8] and small-molecule kinase inhibitors have thus taken the lead as next generation cancer drugs (Table 1) [9]. While this is a significant progress, these inhibitors often interfere with other complex intracellular signaling networks thus causing sometimes severe side effects, and need to be combined with other approaches.

Cells secrete a cocktail of enzymes, such as cholinesterases, peptidases, transpeptidases, nucleotidases, phosphodiesterases, ectokinases, and ectophosphatases, which lead to posttranslational modifications of extracellular matrix (ECM) proteins, and the composition of this cocktail depends on cell type, external stimulations, and disease [10]. Posttranslational modifications of ECM proteins can affect outside-in cell signaling and consequently cell behavior [11]. The massive killing of cancer cells typically increases the local extracellular concentrations of the cytoplasmic content, including ATP, thereby causing additional posttranslational modifications of the ECM. The killing of cancer cells will thus leave behind a “diseased” ECM that can send altered instructive signals to the cells that later invade this cancerous ECM left behind. This has not been considered in the treatment of cancer previously.

Beyond using the concentration of extracellular protein kinases in blood to detect cancer in early stages [12–14], ectokinases and ectophosphatases might serve as new drug targets. Shielded by the plasma membrane, drugs with extracellular targets might cause less side effects as they can less directly interfere with intracellular signaling [15–21]. Even though cancer is not only a disease of cells but also leads to posttranslational modifications of the ECM, the intracellular focus has overshadowed potential extracellular opportunities that could be exploited to address some of these challenges. Here, we thus review

Table 1. Small-molecule kinase inhibitors on the market against kinases.

| Name        | Trade name       | Targeted tyrosine kinase | Disease                                                                 | Producer                      |
|-------------|------------------|--------------------------|-------------------------------------------------------------------------|-------------------------------|
| Imatinib    | Gleevec, Glivec | BCR-Abl                  | Chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs), number of other malignancies | Novartis                      |
| Gefitinib   | Iressa           | EGFR                     | Breast, lung, other cancers                                             | AstraZeneca, Teva              |
| Erlotinib   | Tarceva          | EGFR                     | Nonsmall cell lung cancer (NSCLC), pancreatic cancer, several other types of cancer | Genentech, OSI, Pharmaceuticals, Roche |
| Crizotinib  | Xalkori          | ALK                      | Nonsmall cell lung cancer (NSCLC)                                       | Pfizer                        |
| Dasatinib   | Sprycel          | BCR/ABL and Src family   | Chronic myelogenous leukemia (CML), Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) | Bristol-Myers Squibb          |
| Lapatinib   | Tykerb/Tyverb   | HER2 and EGFR            | Breast cancer, other solid tumors                                       | GlaxoSmithKline               |
| Nilotinib   | Tasigna          | BCR-ABL, KIT, LCK, EPHA3, EPHA8, DDR1, DDR2, PDGFRB, MAPK11, and ZAK | Chronic myelogenous leukemia                                             | Novartis                      |
| Pazopanib   | Votrient         | c-KIT, FGFR, PDGFR, and VEGFR | Renal cell carcinoma, soft tissue sarcoma                               | GlaxoSmithKline               |
| Sunitinib   | Sutent           | PDGFR-Rs, VEGFRs, KIT    | Renal cell carcinoma (RCC), gastrointestinal stromal tumor               | Pfizer                        |
| Sorafenib   | Nexavar          | VEGFR, PDGFR, Raf        | Renal cell carcinoma (RCC), unresectable hepatocellular carcinomas (HCC), thyroid cancer | Bayer, Onyx Pharmaceuticals, AstraZeneca |
| Vandetanib  | Caprelsa         | VEGFR, EGFR, RET-tyrosine kinase | Tumors of the thyroid gland                                              | GlaxoSmithKline               |
| Tofacitinib | Xeljanz, Jakvisus| JAK                      | Rheumatoid arthritis                                                     | Pfizer                        |
| Ruxolitinib | Jakafi, Jakavi   | JAK                      | Myelofibrosis                                                            | Incyte Pharmaceuticals, Novartis |

Current FDA-approved kinase inhibitors on the market in cancer treatment.
the indications that cancer is not only a disease of cells but also of the ECM, and how this newly emerging knowledge of extracellular posttranslational modifications can potentially be exploited for cancer diagnosis and treatment.

**Extracellular Enzymes and Posttranslational Modifications of ECM Coregulate Cancer Progression**

Extracellular strategies are mostly missing although considerable knowledge emerged that the composition and rigidity of the ECM, and consequently ECM cell signaling plays an important role in cancer progression [22, 23]. The first wave of targeting ECM enzymes was motivated by the finding that cancer tissues show upregulated matrix metalloproteinase (MMP) levels, and it was thought that MMP-induced cleavage of ECM would promote the escape of cancer cells from the site of tumors [24, 25] (Fig. 1A). Consequently, MMP inhibitors were designed and went into clinical trials, but with devastating negative outcomes [26, 27]. The main reason for the failure was the lack of appreciation for the complexity of MMP functions and their respective effects on ECM properties and signaling. Only three MMPs had been described at the time when the clinical trials had started, while 23 different MMPs are known today [28]. They were found in different cell types with diverse functions including ECM–cell interactions, cell–cell contact, and regulation of soluble factors among many others [27]. Broad-spectrum inhibitions of MMPs thus interfere with their diverse regulatory roles and thereby cause major side effects [29].

It is thus timely to consider alternate extracellular strategies, including extracellular enzymes or other means by which to regulate posttranslational modifications (Fig. 1B). While the importance of various posttranslational modifications in the ECM are known to regulate cancer progression [23], the significance of ectokinases and ectophosphatases, and the signatures they leave behind, is only now at the verge of being recognized [30]. Why should we even consider extracellular phosphorylation since the ATP levels are typically low in extracellular environment? Extracellular ATP can transiently increase to levels that are sufficiently high to activate ectokinases in those tissues that undergo major necrosis and apoptosis, thereby releasing intracellular content [30]. Also ATP secretion pathways are significantly upregulated in cancers [31, 32] and increased levels of extracellular ATP could recently been measured at tumor sites [33].

Among the reported ectokinases, the most prominent ones are the casein kinase II (CKII) [34], protein kinase A (PKA) [35], protein kinase C (PKC) [36], and the...
recently reported Fam20C kinase [37]. Several ectophosphatases including alkaline phosphatase [38], tartrate-resistant acid phosphatase (TRAP) [39], and the most recently reported PTEN phosphatase [40] have been reported in the ECM. Interestingly, the concentration of the extracellular alkaline phosphatase is already measured routinely as a disease marker in patient’s blood samples to detect liver diseases, bone disorders, or cancer and the TRAP is being discussed as a good candidate [39, 41].

Taken together, considerable evidence is emerging that posttranslational modifications of ECM coregulate cancer progression, that ectokinases and ectophosphatases are found in body fluids of cancer patients, and that kinases can be transiently active in extracellular space in regions where necrosis or other factors cause the release of ATP. As with any discovery, new ideas and strategies are thus beginning to emerge how to exploit these emerging insights into early cancer detection and therapy.

Figure 2. Experimentally verified phosphorylation sites on fibronectin. Schematic representation of plasma fibronectin with modules type I (gray), type II (turquoise), and type III (orange). (A) Locations of various bacterial and cell binding sites on the fibronectin monomer. (B) Experimentally identified phosphorylation sites by mass spectrometry techniques as retrieved from protein data banks Phosida, PhosphoSitePlus, PhosphoNet, HPRD, dbPTM, and UniProt for human Fn (P02751). (C) Locations of protein binding sites on the fibronectin monomer with a special focus on matrix metalloproteinases (MMPs).

Striking Signatures of Extracellular Kinase Activity Are Found in Cancer Tissues

Postulating that massive necrosis might temporarily upregulate ectokinase activity in extracellular space, we recently mined published proteomic data and found a significant upregulation of phosphorylated residues in tissue samples from cancer patients [30]. This included the
phosphorylation of ECM proteins, as well as of cell surface and extracellular domains of transmembrane proteins. Screening more than 60 different extracellular proteins revealed that nearly all can occur in phosphorylated states [30]. Most compelling was the finding that the integrin subunits α4 and β1, two key players in cancer progression and signaling, were found in tissue samples to be phosphorylated in their extracellular domains [30, 42–44]. Since fibronectin [45–47] which is a key component of the ECM is known to be highly upregulated in cancer [48–53], we further analyzed published proteomic data and found that fibronectin is indeed heavily phosphorylated in clinical cancer tissue samples (Fig. 2, Table 2). Heavily phosphorylated regions in fibronectin include and are associated with growth factor binding sites (FnIII4, FnIII13-14) and with domains that regulate fibronectin fibrillogenesis. This is an important finding since growth factor signaling and ECM fibrillogenesis are essential regulators in cancer malignancy and progression [22]. In addition to fibronectin, elevated levels of phosphorylated fibrinogen A are found in the plasma from patients with stage III or IV ovarian cancer compared to healthy controls [54].

Taken together, available data suggest that the upregulated phosphorylation of fibronectin and of some other extracellular proteins is a distinct signature of cancerous ECM. The phosphorylation of the ECM caused by the transient release of ATP by dying cells might thus be physiologically far more important in regulating cancer cell differentiation and tumor progression than previously thought. Indeed, the phosphorylation ratio of peptides increase with tumor size as has been previously shown [13]. Any discovery of new signatures how cancer or cancer tissues are different from the norm might offer valuable entrance points for novel diagnostic or therapeutic strategies. Furthermore, extracellular proteins that are highly phosphorylated in some but not in other cancer types might be suitable as novel markers for the early detection of cancers, or perhaps serve as signature of its malignancy.

**New Strategies for Cancer Diagnostics: Quantification of the Concentrations and Activities of Extracellular Protein Kinases**

One major challenge is to detect cancer in earlier stages in order to treat patients more successfully. According to recent cancer statistics, the 5-year survival rate dramatically drops if cancer is detected at a late stage [55]. Most of the current serum tumor markers are based on the antigen determination method, including CEA, AFP, hCG, PSA, and CA125, but lack tumor specificity and often cannot be used in early cancer
Table 2. Experimentally verified phosphorylation sites on fibronectin in cancer samples.

| Residue (P02751) | Location/binding sites | Reference/databases | Cancer tissues/cells |
|------------------|------------------------|---------------------|----------------------|
| Y101, Y106, T136 | FnI2, Fn–Fn, Heparin, Tenascin, Fibrin | PhosphositePlus, PhosphoNet | In seven patients samples (Y101): ovarian, liver, lung, esophageal, gastric. In one patient sample (Y106): ovarian. In one patient sample (T136): T-cell leukemia. |
| Y372             | FnI1, Collagen, Gelatin | PhosphositePlus, PhosphoNet | In three patient samples: ovarian, liver, hepatocellular carcinoma, hepatocyte–liver |
| Y588             | FnI9, Collagen, Gelatin | PhosphositePlus, PhosphoNet | In one patient sample: lung carcinoma |
| Y641             | FnIII1, Fn–Fn          | PhosphositePlus, PhosphoNet | In one patient sample: pancreatic carcinoma |
| S904             | Linker FnIII3–FnIII4   | PhosphoNet, HPRD, dbPTM [79] | HeLaS3 (cervical cancer) |
| S909             | FnIII4, DNA binding    | HPRD, dbPTM [80]      | Hela cells |
| Y937, T960, S968, T972 | FnIII4, DNA binding | PhosphositePlus, PhosphoNet | In one patient sample (Y037): gastric. In one patient sample (T960): T-cell leukemia. In one patient sample (S968): T-cell leukemia. In one patient sample (T972): T-cell leukemia. Embryonic stem cells. |
| Y1042            | FnIII5                 | PhosphositePlus, PhosphoNet, dbPTM [81] | |
| Y1206            | FnIII7                 | PhosphositePlus, PhosphoNet | In two patients samples: ovarian |
| T1271            | FnIII8, Cell binding region | PhosphositePlus, PhosphoNet | In one patient sample: colorectal (epithelial) |
| T1462            | FnIII10, Cell binding region | PhosphositePlus, PhosphoNet | In one patient sample: pancreatic carcinoma. In one patient sample (T1743): T-cell leukemia. In one patient sample (T1762): esophageal. In one patient sample (T1786): esophageal. In one patient sample (S1833): liver, cholangiocellular carcinoma. In two patient samples (1840): cervical. In one patient sample (T1842): cervical. In two patient samples (T1855): cervical. In one patient sample (T1860): cervical. In one patient sample (Y1879): ovarian. In one patient samples (Y1884): ovarian, T-cell leukemia. |
| Y2258            | FnI11                  | PhosphositePlus, PhosphoNet | In one patient sample: ovarian. |
| S2007            | Variable region IIICS, LDV, REDV integrin binding sites | Phosida [83] | Hela cells |
| S2131, S2139     | FnIII15, Cryptic cysteine | [84] | U266 (immortal B lymphocytes derived from multiple myeloma) |
| S2174            | FnIII15, Cryptic cysteine | Phosida, [83, 85, 86] | Hela cells, HEK, human liver tissue |
| S2182, S2209     | FnIII15, Cryptic cysteine | Phosida [83] | Hela cells |
| S2251            | FnI10, Fibrin binding | Phosida, [84] | U266 (immortal B lymphocytes derived from multiple myeloma) |
| S2258            | FnI11                  | PhosphositePlus, PhosphoNet | In one patient sample: ovarian. |
| S2294            | FnI12, Fibrin binding, Protein-disulfide isomerase binding | Phosida, [83, 85, 86] | Hela cells, HEK, human liver tissue |
| S2295            | FnI12, Fibrin binding, Protein-disulfide isomerase binding | dbPTM [84] | U266 (immortal B lymphocytes derived from multiple myeloma) |
| S2318            | FnI12, Fibrin binding, Protein-disulfide isomerase binding | Phosida, [83, 85, 86] | Hela cells, HEK, Human liver tissue |
| S2328            | FnI12, Fibrin binding, Protein-disulfide isomerase binding | dbPTM, [84] | Serum |
| S2341, S2349     | C-terminus, Disulfide bonds for Fn assembly | PhosphositePlus, PhosphoNet | In two patients samples: breast, ovarian |
| Y2350            | C-terminus, Disulfide bonds for Fn assembly | PhosphositePlus, PhosphoNet | |
| Y2353            | C-terminus, Disulfide bonds for Fn assembly | PhosphoNet, PhosphositePlus [87] | In 12 patient samples, breast, lung, stomach, liver, hepatocellular carcinoma. |
screening and diagnosis [56–63]. To overcome these shortcomings, novel, cheap, and fast diagnostic tools need to be developed. Measurements of ectokinase and ectophosphatase concentrations and activities in serum might thereby provide new opportunities (Fig. 3). Such measurements could be embedded in routinely performed blood tests to screen for cancer long before patients show symptoms. Recent studies with more than 600 patients (374 healthy controls, 229 cancer patients) showed a significant upregulation of ecto-PKA concentrations in serum of cancer patients in contrast to healthy controls [64]. While more than 70% of the control patients had undetectable or low ecto-PKA concentrations in serum, more than 85% of the cancer patients had high levels of PKA concentrations, with average activity fivefold higher compared to the healthy controls. In another independent study, sera of 500 patients (295 various cancers, 155 normal controls, 55 without cancer) were analyzed by autoantibody against ecto-PKA. The presented anti-ecto-PKA measurement showed a 90% sensitivity and 80% specificity compared to the conventional methods with 83% sensitivity and 80% specificity [65]. Only recently, the quantification of ecto-PKA has been patented as a cancer marker for prostate and breast cancer [66]. As suggested by the research group, this approach has the potential to replace the commonly used PSA screening test for prostate cancer and the mammograms screening test for breast cancer, which cost nearly $6 billion annually in the United States alone, with limited reliability of the outcome [67–69]. Alternatively or in combination, the ecto-PKC and ecto-CKII are other kinases well suited for phenotyping as they are reported in the ECM and show upregulated levels in secretory vesicles of prostate cancer samples [36, 70]. Such a path holds considerable promise particularly since a 10-fold increased abundance of ecto-PKC in serum of cancer patients with renal, colon, rectal, adrenal, and lung cancer compared to normal serum has recently been reported [71].

**New Strategies for Cancer Treatment: Drug Targeting of Extracellular Protein Kinases and Phosphatases**

In the last two decades, intracellular protein kinases have emerged as the most important drug targets in pharmaceutical industry leading to some 20 approved drugs on the market and hundreds more in clinical trials [72]. To reduce side effects, a combinatorial approach is needed, one targeting and killing cancer cells while one also tries to prevent or revert the diseased state of ECM. One can further speculate that these drugs might have less side effects as they will not directly interfere with intracellular signaling events [73], but are expected to regulate primarily outside-in cell signaling. Since several important intracellular protein kinases and phosphatase including PKA, PKC, CKII, FAM20C, alkaline phosphatase, and PTEN phosphatase have been found as ectokinases and ectophosphatas, especially in cancer malignancy and progression [30], their potential as novel drug targets has been highlighted [74, 75], but not yet systematically...
exploited. The overexpression of ecto-PKA in secretory vesicles in prostate cancer further points to a putative regulatory role of ectokinases in cancer [70]. The expression of the ecto-PKA kinase, as probed in serum of melanoma patients, correlated with the appearance and size of the tumor and tumor removal reduced the levels of ecto-PKA [14]. Ecto-PKC is another kinase that has been reported to be present and active in sera of cancer patients with renal, colon, rectal, adrenal, and lung cancer [36, 71]. Both ecto-PKC and ecto-CKII have been reported to be expressed in secretory vesicles in prostate cancer and they might thus serve as novel targets [70]. The role for FAM20C kinase [37], which is present and active in the ECM, is already discussed in the regulation of bone metastasis [76].

Besides protein kinases, protein phosphatases could also serve as potential drug targets. Most recently, monoclonal antibodies were designed to target the extracellular alkaline phosphatase that is expressed on the surface of gastrointestinal cancer cells [77]. In addition, the PTEN phosphatase, a tumor suppressor that is known to induce tumor cell death in vitro and in vivo, has been reported to be secreted and subsequently enter other cells where it modifies their signaling and survival [40]. Finally, mutations in PTEN and their down regulation are reported to be involved in invasion and metastasis of colorectal carcinomas, indicating PTEN as a novel drug target and a marker for colorectal carcinoma [78]. Another advantage is that ectokinases and ectophosphatases could be targeted in cases where other drugs are not efficient anymore due to resistance of the tumor. Consequently, selected extracellular protein kinases and phosphatases might be good candidates for the development of novel drug targets.

Future Perspectives

As extracellular protein phosphorylation is moving into the spotlight of attention, our goal here is to stimulate a thinking process how to best utilize this information for the fight of cancer. An increased understanding of the role of ectokinases and ectophosphatases in the regulation of outside-in signaling pathways in cancer malignancy and progression might result not only in exciting new science but also in the design of new combinatorial drugs that can display their functions in extracellular space [15–21], perhaps complementing conventional therapies, by modulating outside-in cell signaling through the posttranslational modification of extracellular proteins. Starting to apply the knowledge gained in the last 60 years about intracellular protein kinases to the extracellular space offers new opportunities. Ultimately, we need to learn not only how to effectively kill cancer cells but also how to repair diseased cancerous ECM that is left behind and has the potency to send altered instructive signals to newly invading cells.

Acknowledgments

This work was supported by the ERC Advanced Grant (European Research Council, Contract Nr. 233157, V. V.), CCMX Competence Centre for Materials Science and Technology, Grant 0-21108-10, the Swiss Initiative in Systems Biology (SystemsX.ch, “Phosphorylation Modulated Networks of the Cell” [PhosphoNetX]), the Swiss National Science Foundation (SNF, Contract Nr. 3100A0-116236), the Swiss National Science Foundation (Postdoctoral fellowship PBEZP3_145998 to G. Y.), and by the ETH Zurich.

Conflict of Interest

None declared.

References

1. Lippert, T. H., H. J. Ruoff, and M. Volm. 2008. Intrinsic and acquired drug resistance in malignant tumors. The main reason for therapeutic failure. Arzneimittelforschung 58:261–264.
2. Gottesman, M. M. 2002. Mechanisms of cancer drug resistance. Annu. Rev. Med. 53:615–627.
3. Ware, K. E., T. K. Hinz, E. Kleczko, K. R. Singleton, L. A. Marek, B. A. Helfrich, et al. 2013. A mechanism of resistance to gefitinib mediated by cellular reprogramming and the acquisition of an FGF2-FGFR1 autocrine growth loop. Oncogenesis 2:e39.
4. Santarpia, M., T. M. De Pas, G. Altavilla, L. Spaggiari, and R. Rosell. 2013. Moving towards molecular-guided treatments: erlotinib and clinical outcomes in non-small-cell lung cancer patients. Future Oncol. 9:327–345.
5. Hunter, T. 2012. Why nature chose phosphate to modify proteins. Philos. Trans. R. Soc. Lond. B Biol. Sci. 367:2513–2516.
6. Ubersax, J. A., and J. E. Ferrell Jr. 2007. Mechanisms of specificity in protein phosphorylation. Nat. Rev. Mol. Cell Biol. 8:530–541.
7. Manning, G., G. D. Plowman, T. Hunter, and S. Sudarsanam. 2002. Evolution of protein kinase signaling from yeast to man. Trends Biochem. Sci. 27:514–520.
8. Holt, L. J., B. B. Tuch, J. Villen, A. D. Johnson, S. P. Gygi, and D. O. Morgan. 2009. Global analysis of Cdk1 substrate phosphorylation sites provides insights into evolution. Science 325:1682–1686.
9. Levitzki, A. 2013. Tyrosine kinase inhibitors: views of selectivity, sensitivity, and clinical performance. Annu. Rev. Pharmacol. Toxicol. 53:161–185.
10. Goding, J. W. 2000. Ecto-enzymes: physiology meets pathology. J. Leukoc. Biol. 67:285–311.
11. Seger, D., R. Seger, and S. Shaltiel. 2001. The CK2 phosphorylation of vitronectin. Promotion of cell adhesion via the alpha(4)beta 3-phosphatidylinositol 3-kinase pathway. J. Biol. Chem. 276:16998–17006.
12. Jaros, J. A., P. C. Guest, H. Ramoune, M. Rothermundt, F. M. Leweke, D. Martins-de-Souza, et al. 2012. Clinical use of phosphorylated proteins in blood serum analysed by immobilised metal ion affinity chromatography and mass spectrometry. J. Proteomics. 76:Spec No.36–42.
13. Kang, J. H., D. Asai, H. Kitzazaki, and Y. Katayama. 2009. Plasma protein kinase C (PKC)alpha as a biomarker for the diagnosis of cancers. Carcinogenesis 30:1927–1931.
14. Kita, T., J. Goydos, E. Reitman, R. Ravatn, Y. Lin, W. C. Shih, et al. 2004. Extracellular cAMP-dependent protein kinase (ECPKA) in melanoma. Cancer Lett. 208:187–191.
15. Dogan, S. S., and B. Esmaeli. 2009. Ocular side effects associated with imatinib mesylate and perifosine for gastrointestinal stromal tumor. Hematol. Oncol. Clin. North Am. 23:109–114, ix.
16. Lee, M. W., C. W. Seo, S. W. Kim, H. J. Yang, H. W. Lee, J. H. Choi, et al. 2004. Cutaneous side effects in non-small cell lung cancer patients treated with Iressa (ZD1839), an inhibitor of epidermal growth factor. Acta Derm. Venereol. 84:23–26.
17. Sipples, R. 2006. Common side effects of anti-EGFR therapy: acneform rash. Semin. Oncol. Nurs. 22:28–34.
18. Nelson, V., J. Ziehr, M. Agulnik, and M. Johnson. 2013. Afatinib: emerging next-generation tyrosine kinase inhibitor for NSCLC. Onco. Targets Ther. 6:135–143.
19. Choueiri, T. K. 2008. Axitinib, a novel anti-angiogenic drug with promising activity in various solid tumors. Curr. Opin. Investig. Drugs 9:658–671.
20. Weickhardt, A. J., M. S. Rothman, S. Salian-Mehta, K. Kiseljak-Vassiliades, A. B. Oton, R. C. Doebele, et al. 2012. Rapid-onset hypogonadism secondary to crizotinib use in men with metastatic nonsmall cell lung cancer. Cancer 118:5302–5309.
21. Hartmann, J. T., M. Haap, H. G. Kopp, and H. P. Lipp. 2009. Tyrosine kinase inhibitors—a review on pharmacology, metabolism and side effects. Curr. Drug Metab. 10:470–481.
22. Lu, P., V. M. Weaver, and Z. Werb. 2012. The extracellular matrix: a dynamic niche in cancer progression. J. Cell Biol. 196:395–406.
23. Leeming, D. J., A. C. Bay-Jensen, E. Vassiliadis, M. R. Larsen, K. Henrikson, and M. A. Karsdal. 2011. Post-translational modifications of the extracellular matrix are key events in cancer progression: opportunities for biochemical marker development. Biomarkers 16:193–205.
24. Egeblad, M., and Z. Werb. 2002. New functions for the matrix metalloproteinases in cancer progression. Nat. Rev. Cancer 2:161–174.
25. Zucker, S., and J. Vacirca. 2004. Role of matrix metalloproteinases (MMPs) in colorectal cancer. Cancer Metastasis Rev. 23:101–117.
26. Overall, C. M., and C. Lopez-Otin. 2002. Strategies for MMP inhibition in cancer: innovations for the post-trial era. Nat. Rev. Cancer 2:657–672.
27. Goussens, L. M., B. Fingleton, and L. M. Matrisian. 2002. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 295:2387–2392.
28. Kessenbrock, K., V. Plaks, and Z. Werb. 2010. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 141:52–67.
29. Sang, Q. X., Y. Jin, R. G. Newcomer, S. C. Monroe, X. Fang, D. R. Hurst, et al. 2006. Matrix metalloproteinase inhibitors as prospective agents for the prevention and treatment of cardiovascular and neoplastic diseases. Curr. Top. Med. Chem. 6:289–316.
30. Yalak, G., and V. Vogel. 2012. Extracellular catalytic subunit activity of the extracellular protein kinase (ECPKA) as a biomarker for the diagnosis of cancers. Carcinogenesis 30:1927–1931.
HL-60 cells is induced by retinoic acid. J. Cell. Biochem. 76:420–436.

39. Yamada, S., K. Tsuruya, H. Yoshida, M. Taniguchi, N. Haruyama, S. Tanaka, et al. 2013. The clinical utility of serum tartrate-resistant acid phosphatase 5b in the assessment of bone resorption in patients on peritoneal dialysis. Clin. Endocrinol. 78:844–851.

40. Hopkins, B. D., B. Fine, N. Steinbach, M. Dendy, Z. Rapp, J. Shaw, et al. 2013. A secreted PTEN phosphatase that enters cells to alter signaling and survival. Science 341:399–402.

41. Wiwanitkit, V. 2001. High serum alkaline phosphatase levels, a study in 181 Thai adult hospitalized patients. BMC Fam. Pract. 2:2.

42. Desgrosellier, J. S., and D. A. Cheresh. 2010. Integrins in cancer: biological implications and therapeutic opportunities. Nat. Rev. Cancer 10:9–22.

43. Yao, E. S., H. Zhang, Y. Y. Chen, B. Lee, K. Chew, D. Moore, et al. 2007. Increased beta1 integrin is associated with decreased survival in invasive breast cancer. Cancer Res. 67:659–664.

44. Nikolopoulos, S. N., P. Blaikie, T. Yoshioka, W. Guo, and F. G. Giancotti. 2004. Integrin beta4 signaling promotes tumor angiogenesis. Cancer Cell 6:471–483.

45. Pankov, R., and K. Yamada. 2002. Fibronectin at a glance. J. Cell Biol. 155:3861–3863.

46. Hynes, R. O. 2009. The extracellular matrix: not just pretty. Curr. Opin. Cell Biol. 21:346–354.

47. Vogel, V. 2006. Mechanotransduction involving multimodular proteins: converting force into biochemical signals. Annu. Rev. Biophys. Biomol. Struct. 35:459–488.

48. Bissell, D. M. 2001. Chronic liver injury, TGF-beta, and cancer. Exp. Cell Res. 268:293–302.

49. Helleman, J., M. P. Jansen, K. Ruigrok-Ritstier, I. L. van Staveren, M. P. Look, M. E. van Meijer-Gelder, et al. 2008. Association of an extracellular matrix gene cluster with breast cancer prognosis and endocrine therapy response. Clin. Cancer Res. 14:5535–5544.

50. Hynes, R. O. 2007. Cell-matrix adhesion in vascular development. J. Thromb. Haemost. 5:32–40.

51. Zeng, Z. Z., Y. Jia, N. J. Hahn, S. M. Markwart, K. F. Rockwood, and D. L. Livant. 2006. Role of focal adhesion kinase and phosphatidylinositol 3'-kinase in integrin fibronectin receptor-mediated, matrix metalloproteinase-1-dependent invasion by metastatic prostate cancer cells. Cancer Res. 66:8091–8099.

52. Ricciardelli, C., and R. J. Rodgers. 2006. Extracellular matrix of ovarian tumors. Semin. Reprod. Med. 24:270–282.

53. Fornaro, M., J. Plescia, S. Chheang, G. Tallini, Y. M. Zhu, M. King, et al. 2003. Fibronectin protects prostate cancer cells from tumor necrosis factor-alpha-induced apoptosis via the AKT/survivin pathway. J. Biol. Chem. 278:50402–50411.

54. Ogata, Y., C. J. Heppelmann, M. C. Charlesworth, B. J. Madden, M. N. Miller, K. R. Kalli, et al. 2006. Elevated levels of phosphorylated fibrinogen-alpha-isoforms and differential expression of other post-translationally modified proteins in the plasma of ovarian cancer patients. J. Proteome Res. 5:3318–3325.

55. Siegel, R., D. Naishadham, and A. Jemal. 2013. Cancer statistics, 2013. CA Cancer J. Clin. 63:11–30.

56. Molina, R., V. Barak, A. van Dalen, M. J. Duffy, R. Einarssson, M. Gion, et al. 2005. Tumor markers in breast cancer- European Group on Tumor Markers recommendations. Tumour Biol. 26:281–293.

57. Leonard, G. D., J. A. Low, A. W. Berman, and S. M. Swain. 2004. CA 125 elevation in breast cancer: a case report and review of the literature. Breast J. 10:146–149.

58. Tricoli, J. V., M. Schoenfeldt, and B. A. Conley. 2004. Detection of prostate cancer and predicting progression: current and future diagnostic markers. Clin. Cancer Res. 10:3943–3953.

59. Bremnes, R. M., R. Sirera, and C. Camps. 2005. Circulating tumour-derived DNA and RNA markers in blood: a tool for early detection, diagnostics, and follow-up? Lung Cancer 49:1–12.

60. Qin, L. X., and Z. Y. Tang. 2004. Recent progress in predictive biomarkers for metastatic recurrence of human hepatocellular carcinoma: a review of the literature. J. Cancer Res. Clin. Oncol. 130:497–513.

61. Tarro, G., A. Perna, and C. Esposito. 2005. Early diagnosis of lung cancer by detection of tumor liberated protein. J. Cell. Physiol. 203:1–5.

62. Crawford, N. P., D. W. Collier, and S. Galandiuk. 2003. Tumor markers and colorectal cancer: utility in management. J. Surg. Oncol. 84:239–248.

63. Gretzer, M. B., and A. W. Partin. 2003. PSA markers in prostate cancer detection. Urol. Clin. North Am. 30:677–686.

64. Wang, H., M. Li, W. Lin, W. Wang, Z. Zhang, E. R. Rayburn, et al. 2007. Extracellular activity of cyclic AMP-dependent protein kinase as a biomarker for human cancer detection: distribution characteristics in a normal population and cancer patients. Cancer Epidemiol. Biomarkers Prev. 16:789–795.

65. Nesterova, M. V., N. Johnson, C. Cheadle, S. E. Bates, S. Mani, C. A. Stratakis, et al. 2006. Autoantibody biomarker: extracellular protein kinase A. Cancer Res. 66:8971–8974.

66. Fushak, R., and D. Held. 2013. Measurement of PKA for cancer detection. Patent. US_8455200_B2

67. Smith, D. S., P. A. Humphrey, and W. J. Catalona. 1997. The early detection of prostate carcinoma with prostate specific antigen: the Washington University experience. Cancer 80:1852–1856.
68. Elmore, J. G., K. Armstrong, C. D. Lehman, and S. W. Fletcher. 2005. Screening for breast cancer. JAMA 293:1245–1256.

69. Harris, L., H. Fritsche, R. Mennel, L. Norton, P. Ravdin, S. Taube, et al. 2007. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J. Clin. Oncol. 25:5287–5312.

70. Babiker, A. A., G. Ronquist, B. Nilsson, and K. N. Ekdahl. 2006. Overexpression of ecto-protein kinases in prostasomes of metastatic cell origin. Prostate 66:675–686.

71. Cho, Y. S., Y. G. Park, Y. N. Lee, M. K. Kim, S. Bates, L. Tan, et al. 2000. Extracellular protein kinase A as a cancer biomarker: its expression by tumor cells and reversal by a myristate-lacking Ca and RIIb subunit overexpression. Proc. Natl Acad. Sci. USA 18:835–840.

72. Cohen, P., and D. R. Alessi. 2013. Kinase drug discovery–what’s next in the field? ACS Chem. Biol. 8:96–104.

73. Ehrlich, Y. H., and E. Kornecki. 1999. Ecto-protein kinases as mediators for the action of secreted ATP in the brain. Prog. Brain Res. 120:411–426.

74. Redegeld, F. A., C. C. Caldwell, and M. V. Sitkovsky. 1999. Ecto-protein kinases: ecto-domain phosphorylation as a novel target for pharmacological manipulation? Trends Pharmacol. Sci. 20:453–459.

75. Wright, X., and J. F. Wright. 2013. Biological insights into therapeutic protein modifications throughout trafficking and their biopharmaceutical applications. Int. J. Cell Biol. 2013:273086.

76. Guo, X., and X. F. Wang. 2012. New secrets behind bone metastasis. Cell Res. 22:1309–1311.

77. Li, M., J. Gao, R. Feng, Y. Wang, X. Chen, J. Sun, et al. 2013. Generation of monoclonal antibody MS17-57 targeting secreted alkaline phosphatase ectopically expressed on the surface of gastrointestinal cancer cells. PLoS One 8:e77398.

78. Li, X. H., H. C. Zheng, H. Takahashi, S. Masuda, X. H. Yang, and Y. Takano. 2009. PTEN expression and mutation in colorectal carcinomas. Oncol. Rep. 22:757–764.

79. Daub, H., J. V. Olsen, M. Bairlein, F. Gnadt, F. S. Oppermann, R. Korner, et al. 2008. Kinase-selective enrichment enables quantitative phosphoproteomics of the kinome across the cell cycle. Mol. Cell 31:438–448.

80. Sugiyama, N., T. Masuda, K. Shinoda, A. Nakamura, M. Tomita, and Y. Ishihama. 2007. Phosphopeptide enrichment by aliphatic hydroxy acid-modified metal oxide chromatography for nano-LC-MS/MS in proteomics applications. Mol. Cell Proteomics 6:1103–1109.

81. Rigbolt, K. T., T. A. Prokhorova, V. Akimov, J. Henningsen, P. T. Johansen, I. Kratchmarova, et al. 2011. System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation. Sci. Signal. 4:rs3.

82. Goswami, T., and B. Bryan. 2011. Methods for the isolation of phosphoproteins and phosphopeptides for mass spectrometry analysis: toward increased functional phosphoproteomics. Sample Preparation in Biological Mass Spectrometry 2011, pp 627–655 Date: 20 May 2011.

83. Olsen, J. V., B. Blagoev, F. Gnadt, B. Macek, C. Kumar, P. Mortensen, et al. 2006. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell 127:635–648.

84. Zhou, W., M. M. Ross, A. Tessitore, D. Ornstein, A. Vanmeter, L. A. Liotta, et al. 2009. An initial characterization of the serum phosphoproteome. J. Proteome Res. 8:5523–5531.

85. Ruse, C. I., D. B. McClatchy, B. Lu, D. Cociorva, A. Motoyama, S. K. Park, et al. 2008. Motif-specific sampling of phosphoproteomes. J. Proteome Res. 7:2140–2150.

86. Han, G., M. Ye, H. Liu, C. Song, D. Sun, Y. Wu, et al. 2010. Phosphoproteome analysis of human liver tissue by long-gradient nanoLC coupled with multiple stage MS analysis. Electrophoresis 31:1080–1089.

87. Rikova, K., A. Guo, Q. Zeng, A. Possemato, J. Yu, H. Haack, et al. 2007. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell 131:1190–1203.

88. Lee, H. J., K. Na, M. S. Kwon, H. Kim, K. S. Kim, and Y. K. Paik. 2009. Quantitative analysis of phosphopeptides in search of the disease biomarker from the hepatocellular carcinoma specimen. Proteomics 9:3395–3408.

89. Bennetzen, M. V., D. H. Larsen, J. Bunkenborg, J. Bartek, J. Lukas, and J. S. Andersen. 2010. Site-specific phosphorylation dynamics of the nuclear proteome during the DNA damage response. Mol. Cell Proteomics 9:1314–1323.

90. Han, G., M. Ye, H. Zhou, X. Jiang, S. Feng, X. Jiang, et al. 2008. Large-scale phosphoproteome analysis of human liver tissue by enrichment and fractionation of phosphopeptides with strong anion exchange chromatography. Proteomics 8:1346–1361.