R&D Systems Cancer Focus
spring | 2006

Cancer FOCUS

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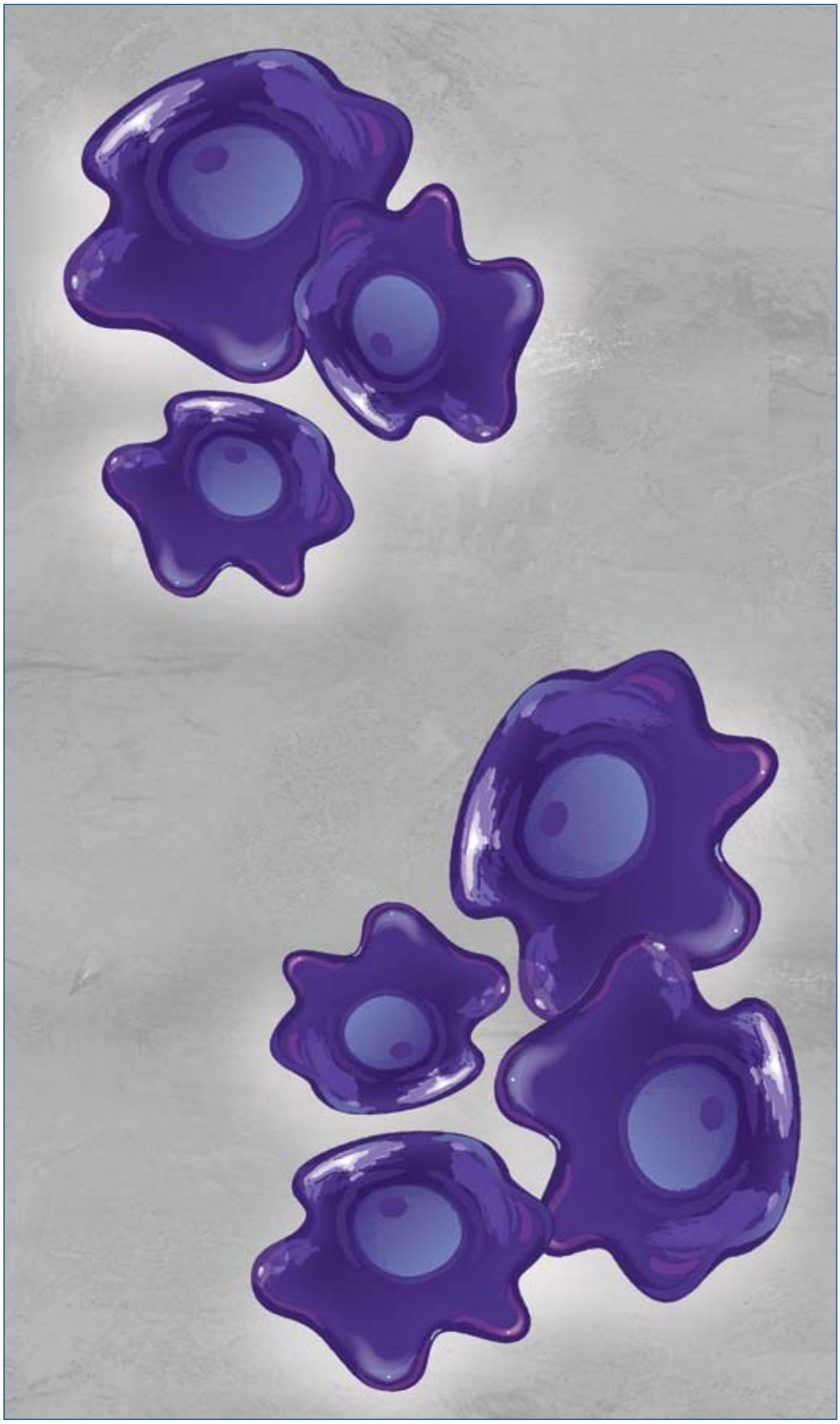


R&D SYSTEMS
Quality | Selection | Performance | Results

ErbB Family of Receptor Tyrosine Kinases

As the first receptor tyrosine kinase (RTK) to be discovered, the epidermal growth factor receptor (EGF R; also known as ErbB1 and HER1) has helped establish many of the principles of RTK function. EGF R forms an RTK subfamily with three other closely related receptors: the orphan receptor ErbB2 (HER2); the impaired kinase ErbB3 (HER3); and ErbB4 (HER4). ErbB family members are essential regulators of cell proliferation, differentiation, migration, and metabolism. Alterations in ErbB activation and expression have been implicated in numerous human malignancies, including breast cancer, lung cancer, and glioblastoma.

RTKs contain an extracellular ligand-binding domain connected through a single transmembrane helix to a cytoplasmic kinase domain. In addition to the conserved protein tyrosine kinase core, an RTK's cytoplasmic domain contains regulatory regions subject to tyrosine autophosphorylation. These autophosphorylation sites on ErbB family members and other RTKs provide a mechanism for the recognition and assembly of signaling complexes, functioning as binding sites for Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains present on a variety of recruited signaling proteins. Proteins recruited by the ErbB family include upstream components of the Raf/MEK/ERK and PI 3-kinase/PDK1/Akt signaling cascades.

RTK-Related Products

ANALYTE	ANTIBODIES	ELISAs/ASSAYS	PROTEINS
Axl	H M	H M	H M
C1q R1/CD93	H M		
DDR1	H		
DDR2	H		
Dtk	H M	H M	H M
EGF R	H M	H	H
EphA1	H		H M
EphA2	M		H M
EphA3	M		M
EphA4	M		M
EphA5	R		H M R
EphA6	M		M
EphA7	M		M
EphA8	M		M
EphB1	R		R
EphB2	M		M
EphB3	M		M
EphB4	M		H M
EphB6	M		M
ErbB2	H	H	H
ErbB3	H	H	H
ErbB4	H	H	H
FGF R1	H		H
FGF R2	H M	H	H M
FGF R3	H M	H	H M
FGF R4	H M		H
FGF R5	H M		
Flt-3	H M		H M
HGF R	H M	H M	H M
IGF-I R	H	H	H
IGF-II R	H		H
INSRR	H		
Insulin R/CD220	H	H	H
M-CSF R	H	H	H
Mer	H M		H M
MSP R/Ron	H M		H M
MuSK	R		
PDGF R α	H M	H	H M
PDGF R β	H M	H	H M
Ret	H M		H M
ROR1	H		
ROR2	H		
SCF R/c-kit	H M	H	H
Tie-1	H		H
Tie-2	H M Z	H M	H M Z
TrkA	H R	H	H R
TrkB	H M		H M
TrkC	H M		H M
VEGF R1/Flt-1	H M	H M	H M
VEGF R2/Flk-1	H M	H M	H M
VEGF R3/Flt-4	H M	H M	H M

Key: H Human M Mouse R Rat Z Zebrafish

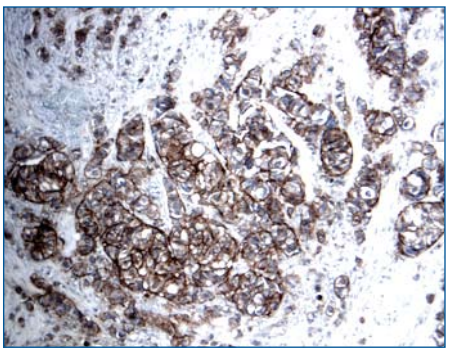


Figure 1. Immunohistochemical detection of phosphorylated ErbB2 in paraffin-embedded human breast cancer tissue sections using R&D Systems rabbit anti-human phospho-ErbB2 (Y1248) affinity purified polyclonal antibody (Catalog # AF1768). Tissues were stained using R&D Systems anti-rabbit HRP-DAB Cell and Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue).

Phospho-Specific RTK Antibodies

ANALYTE	SPECIES	CATALOG #
EGF R (Y1173)	H	AF1095
ErbB2 (Y1248)	H	AF1768
Flt-3 (Y591)	H	AF368
HGF R (Y1234/5)	H	AF2480
Insulin R (Y1162/3)	H	AF2507
PDGF R α (Y742)	H	AF2114
PDGF R α (Y762)	H	AF21141
PDGF R β (Y751)	H	AF1767
PDGF R β (Y1021)	H	AF2316
Tie-2 (Y992)	H M	AF2720
Trk A (Y490)	H	AF2578
VEGF R2/Flk-1 (Y1214)	H	AF1766

PROTEOME PROFILER™ ANTIBODY ARRAYS	CATALOG #
Human Phospho RTK Array Kit	ARY001
Human Phospho MAPK Array Kit	ARY002

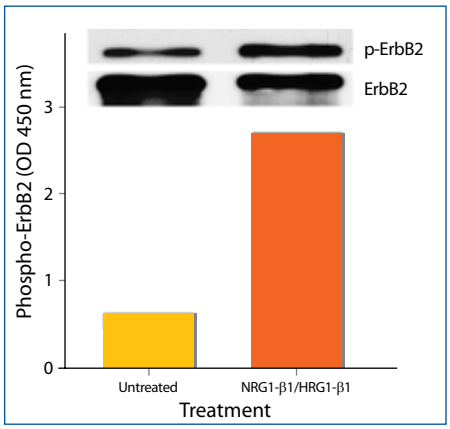


Figure 2. R&D Systems Human Phospho-ErbB2 DuoSet® IC ELISA Kit (Catalog # DYC1768) was used to detect ligand-induced tyrosine phosphorylation. MDA-MB-453 human breast cancer cells were untreated or treated with 100 ng/mL R&D Systems Recombinant Human NRG1-β1/HRG1-β1 (Catalog # 396HB). Cell lysates were analyzed by ELISA and by IP-Western blot (inset). IP-Western blots for phospho-ErbB2 were performed using R&D Systems anti-human ErbB2 monoclonal antibody (Catalog # MAB1129). Total ErbB2 was detected on the same blot using goat anti-human ErbB2 biotinylated, affinity purified polyclonal antibody (R&D Systems Catalog # BAF1129). Bands were visualized with Streptavidin-HRP (R&D Systems Catalog # DY998) followed by chemiluminescent detection with WesternGlo™ substrate (R&D Systems Catalog # AR004).

Genotoxic Stress Response: DNA Damage

The human genome is exposed to potentially deleterious genotoxic events during every cell division cycle. This endogenous source of DNA damage results from cellular metabolism or routine errors in DNA replication and recombination. In addition, cellular and organismal exposure to exogenous genotoxic agents such as ultraviolet light, oxidative stress, and chemical mutagens, leads to a variety of nucleotide modifications and DNA strand breaks. In order to combat these attacks on the genome, the cell has evolved a response system that induces cell cycle arrest to allow sufficient time to repair the incurred damage. The DNA damage response system also activates the appropriate DNA repair pathway, or, in the case of irreparable damage, induces apoptosis.

In the last decade, the characterization of many proteins involved in sensing and responding to DNA damage has enhanced our understanding of these genotoxic stress responses. In addition, mutation in the genes that encode DNA damage response proteins can result in a number of genomic instability syndromes. Genomic instability syndromes are autosomal recessive disorders that result in a heightened predisposition to multiple types of cancer. Thus, the significance of the genotoxic stress response is indicated by disease in the absence of critical proteins that sense, relay, or transduce the signal.

Duoset® IC IP Kinase Assays		
ANALYTE	SPECIES	CATALOG #
Chk1	H M R	DYC1630
Chk2	H M R	DYC1358

Genotoxic Stress Response		
ANALYTE	ANTIBODIES	ELISAs / ASSAYS
APE	H M R Ms	
ATM	H M R	H
ATRIP	H M R	
BARD1	H M R	
BRCA1	H M R	
BRCA2	H	
CBP	H M R	
Chk1	H M R	H M R
Chk2	H M R	H M R
H2AX	H	
MDM2	H M R	
Mre11	H	
Nbs1	H M R	
NTH1	H	
p21/CIP1/CDKN1A	H	H
p27/Kip1	H M R	H
p53	H M R	H M
Pin1	H M R	
Rad1	H	
Rad17	H R	H
SMC1	H	
UNG	H	

Key: H Human M Mouse Ms Multi-Species R Rat

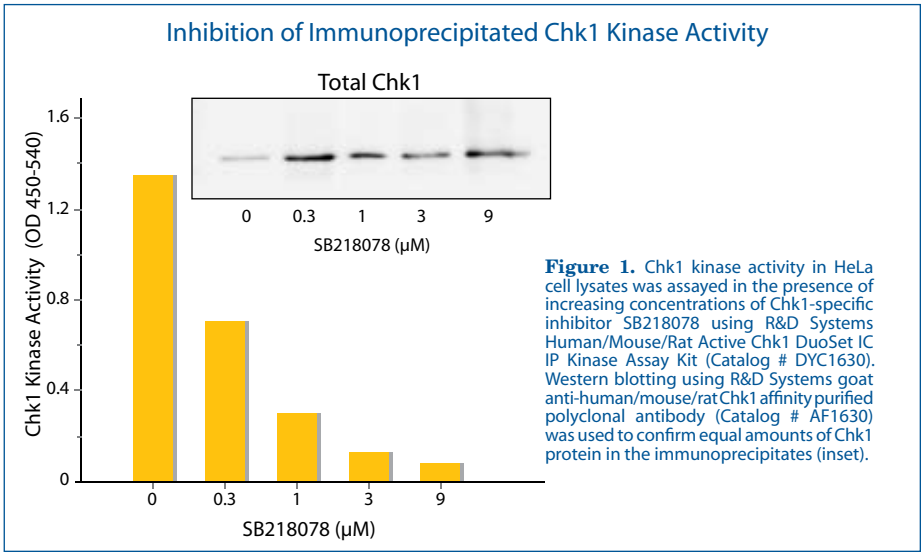


Figure 1. Chk1 kinase activity in HeLa cell lysates was assayed in the presence of increasing concentrations of Chk1-specific inhibitor SB218078 using R&D Systems Human/Mouse/Rat Active Chk1 DuoSet IC IP Kinase Assay Kit (Catalog # DYC1630). Western blotting using R&D Systems goat anti-human/mouse/rat Chk1 affinity purified polyclonal antibody (Catalog # AF1630) was used to confirm equal amounts of Chk1 protein in the immunoprecipitates (inset).

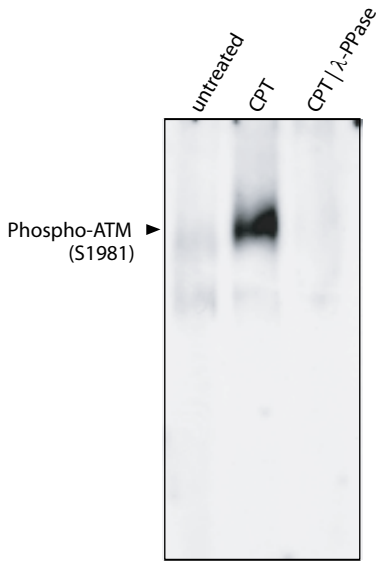


Figure 2. Western blot using R&D Systems rabbit anti-human phospho-ATM (S1981) affinity purified polyclonal antibody (Catalog # AF1655) to detect phosphorylation of ATM (Ataxia Telangiectasia Mutated). HeLa cells were untreated (left lane), treated with the Topoisomerase I inhibitor, camptothecin (CPT; middle lane), or treated with CPT and λ-phosphatase (right lane). This antibody also recognizes the comparable phosphorylated sites in mouse (S1987) and rat (S1952) ATM.

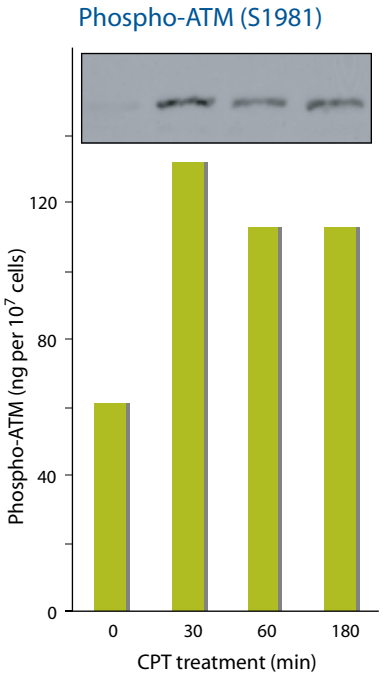


Figure 3. Quantification of phospho-ATM in human osteosarcoma U2-OS cells using R&D Systems Human phospho-ATM (S1981) DuoSet IC ELISA Kit (Catalog # DYC1655). Cells were left untreated or treated with camptothecin for the indicated time prior to cellular extract preparation. The same cellular extracts were immunoblotted (inset) with R&D Systems rabbit anti-human phospho-ATM (S1981) affinity purified polyclonal antibody (Catalog # AF1655). The DuoSet IC results correlate well with the Western blot data.

Angiogenesis

Angiogenesis, or the process of new blood vessel formation, is a natural event that occurs under both normal and pathological conditions. In the normal state, two distinct processes can be seen. One process utilizes endothelial progenitor cells. These are usually derived from bone marrow and initiate endothelial growth and vascular tube formation. The second process utilizes existing vasculature to generate new vessels, and is highly dependent on endothelial cell activation and protease secretion. Under pathological conditions, many of the same steps involved in normal vessel formation are repeated. However, the structures formed are often functionally abnormal, possibly due to an imbalance in the angiogenic process. Multiple factors contribute to angiogenesis, including soluble growth and differentiative factors, extracellular matrix components, membrane-bound receptors, and intracellular signaling molecules. R&D Systems has an extensive and diverse offering of reagents for studying proteins that are known to be involved in both angiogenesis and its natural counterpart, anti-angiogenesis.

Pro-Angiogenic

ANALYTE	ANTIBODIES	ELISAs/ASSAYS	PROTEINS
Angiopoietins	H	H	H
Collagen I			R B
EGF	H M	H	H M R
Erythropoietin	H M	H M	H M R
FGFs	H M B	H	H M B
Fibronectin	H		H B
CX3CL1/Fractalkine	H M R	H M R	H M R
GM-CSF	H M R Ca F P	H M R F	H M R Ca F P
HGF	H M	H	H M
HIF-1 α	H M R	H M	
IGF-I	H M	H M	H M
IL-6	H M R Ca CR E P	H M R Ca P	H M R Ca CR E F P
CXCL8/IL-8	H Ca F P	H P	H Ca F P
IL-13	H M R	H M	H M R Pr
CCL2/MCP-1	H M Ca CR	H M Ca	H M R Ca
MMPs	H M	H M	H M
PD-ECGF	H		H
PDGF-B	H Ms	H M R	H R
PGE2		Ms	
PIGF	H	H	H
CXCL12/SDF-1	H M	H M	H M F RM
Tenascins	H M R		
TGF-α	H	H	H
TGF-β1	Ms	H M R Ca P	H P
uPA	H		H
VEGFs	H M R Ca Z	H M R	H M R Ca Z

Anti-Angiogenic

ANALYTE	ANTIBODIES	ELISAs/ASSAYS	PROTEINS
Angiostatin	H		
CXCL14/BRAK	H M	H	H M
N-Cadherin			H
CCR2	H		
CD44	H		
Endostatin	H M	H	
Eph Bs	M R		H M R
Ephrin-A1	M		M
FGF R1	H		H
ICAM-1/CD54	H M R	H M R	H M R
IFN-α	H M CR P	H M	H M R CR F P Pr
IL-4	H M R Ca CR E F P	H M R CR F P	H M R B Ca CR E F P Pr
Integrins	H M		H
CXCL10/IP-10/CRG-2	H M CR	H M	H M CR
LIF	H M	H M	
MMP-12	H		H
CXCL4/PF4	H M	H M	H M
E-Selectin	H M R	H M	H M R
Serpin F1	H M		
SPARC	H M		
Thrombospondins	H		
Ties	H M Z	H M	H M Z
TIMPs	H M R	H M R	H M R
TL1A/TNFSF15	H		H M
VCAM-1	H M	H M	H M
VEGF Rs	H M	H M	H M

Key: B Bovine Ca Canine CR Cotton Rat E Equine F Feline H Human M Mouse Ms Multi-species P Porcine Pr Primate R Rat RM Rhesus/Macaque Z Zebrafish

For a more complete listing of angiogenesis related products please visit our website at: **www.RnDSystems.com/go/Angiogenesis**

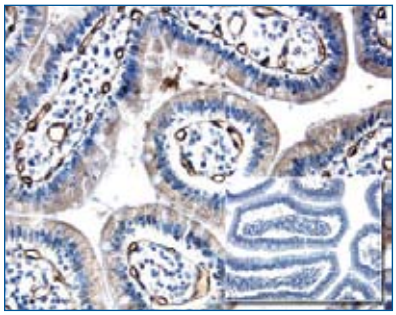


Figure 1. Detection of Angiopoietin-2 in paraffin-embedded human gastrointestinal cancer tissue sections using R&D Systems goat anti-human Angiopoietin-2 affinity purified polyclonal antibody (Catalog # AF623). Tissues were stained with R&D Systems anti-goat HRP-DAB Cell and Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Inset shows control staining without primary antibody.

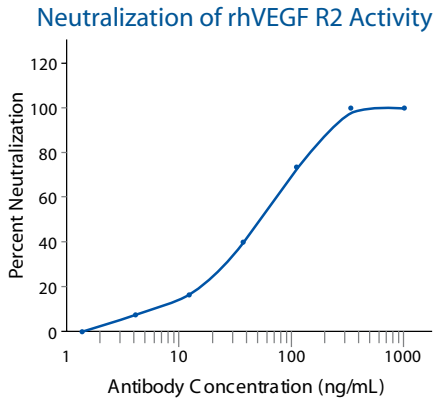


Figure 2. Human VEGF R2/Fc chimera (R&D Systems Catalog # 357-KD) inhibits hVEGF stimulated ^{3}H-thymidine incorporation by human umbilical vein endothelial cells (data not shown). This activity is blocked by addition of R&D Systems mouse anti-human VEGF R2 monoclonal antibody (Catalog # MAB3572).

Tumor Markers

Tumor markers are endogenous proteins or metabolites whose amounts or modifications are indicative of tumor state, progression characteristics, and response to therapies. They are present in tumor tissues or body fluids and encompass a wide variety of molecules, including transcription factors, cell surface receptors, and secreted proteins. Effective tumor markers are in great demand since they have the potential to reduce cancer mortality rates by facilitating diagnosis of cancers at early stages and by helping to individualize treatments. During the last decade, improved understanding of carcinogenesis and tumor progression has revealed a large number of potential tumor markers. It is predicted that even more will be discovered in the near future with the application of current technologies such as tissue microarrays, antibody arrays, and mass spectrometry. To apply these discoveries to patient care, vigorous validation and assay development for many tumor markers is currently underway. R&D Systems has a wide range of research reagents for use at every stage of the tumor marker development process. These products include a large collection of recombinant proteins, antibodies, and protein quantification kits (Quantikine® ELISA kits and DuoSet® ELISA development kits).

Cancer Biomarkers

MOLECULE	ANTIBODIES	ELISAs/ASSAYS	PROTEINS
A33	M		
α-Fetoprotein	H M	H	
Aurora A	H		
Bcl-2	H M R	H	H
E-Cadherin	H M	H	H M
Carbonic Anhydrase IX	H M		H M
β-Catenin	H M R X	H	
Cathepsin D	H M		H M
CD44	H		
EGF	H M	H	H M R
EGF R	H M	H	H
ErbB2	H	H	H
ER α/NR3A1	H		
ER β/NR3A2	H		
FGF acidic	H B	H	H B
FGF basic	H B	H	H M B
Galectin-3	H M	M	H M
Glypican 3	H		H
HIN-1/Secretoglobulin 3A1	H M		
IGF-I	H M	H M	H M
IGFBP-3	H M	H M	H M
IL-6	H M R Ca CR E P	H M R Ca P	H M R Ca CR E F P
Kallikrein 3/PSA	H	H	H
Kallikrein 6/Neurosin	H		
M-CSF	H M	H M	H M
MMP-2, -3, & -9	H M	H M	H M
Nestin	H R		
NG2/MCSP	H		
Osteopontin	H M	H M	H M B
p21/CIP1	H	H	
p27	H M R		
p53	H M R	H M	
Progesterone R/NR3C3	H		
Prolactin	H M		H M
S100B	H		
SCF R/c-kit	H M	H	H
Serpin E1/PAI-1	H		H
Serum Amyloid A1 (SAA1)	M		
Survivin	H	H	H
TIMP-1	H M R	H M R	H M R
TIMP-2 & -4	H	H	H
TIMP-3	H		H
TNF-α	H M R B Ca CR E RM	H M R Ca E P Pr RM	H M R Ca E F P Pr RM
TRAF-4	H		
uPA	H		H
uPAR	H M	H	H M
VCAM-1	H M	H M	H M
VEGF	H M R Ca Z	H M R	H M R Ca Z

Key: B Bovine Ca Canine CR Cotton Rat E Equine F Feline H Human M Mouse P Porcine Pr Primate R Rat RM Rhesus/Macaque X *Xenopus* Z Zebrafish

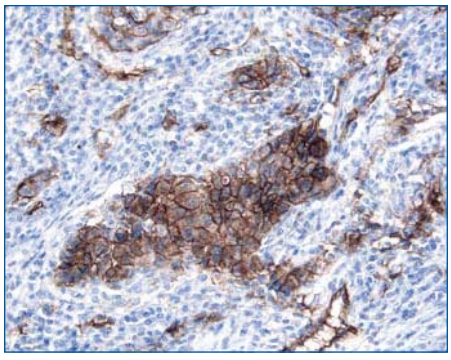


Figure 1. Detection of Kallikrein 6 in paraffin-embedded breast cancer tissue sections using R&D Systems goat anti-human Kallikrein 6 affinity-purified polyclonal antibody (Catalog # AF2008). Tissues were stained using R&D Systems anti-goat HRP-DAB Cell and Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue).

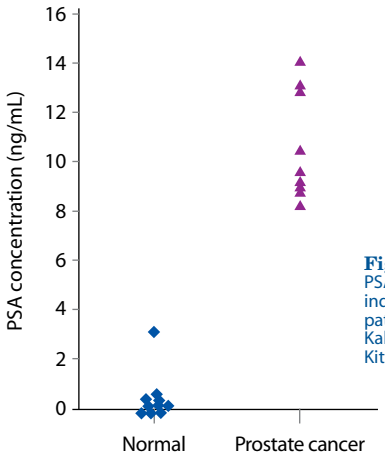


Figure 2. Measurement of serum PSA concentrations in 10 normal individuals and 10 prostate cancer patients using R&D Systems Human Kallikrein 3/PSA Quantikine ELISA Kit (Catalog # DKK300).

Cell Adhesion in Tumor Progression & Metastasis

Adhesion proteins have been identified as markers of tumor cell invasiveness and tumor progression by gain or loss of expression or function. Cell signaling can be activated "outside-in" through engagement of adhesion molecules, or "inside-out" through signals which modify the status of adhesion proteins. Both processes link the status of integrins and other adhesion proteins with changes in signaling status of tumor cells. Cadherin switching from E-cadherin to N-cadherin indicates an epithelial-to-mesenchymal transition associated with tumor progression. Expression, activity, and turnover of adhesion molecules directly affect the ability of tumor cells to escape contact-mediated growth inhibition, as well as dislodge, travel, extravasate, and establish metastatic growth. R&D Systems offers a wide variety of reagents for studying these processes.

Cultrex® Invasion Assays	CATALOG #
Laminin I	3456-096-K
Collagen IV	3458-096-K
Collagen I	3457-096-K
Basement Membrane Extract	3455-096-K
Cultrex® 3D Culture Matrix™	
Rat Collagen I	3447-020-01
Laminin I	3446-005-01
Basement Membrane Extract	3445-048-01

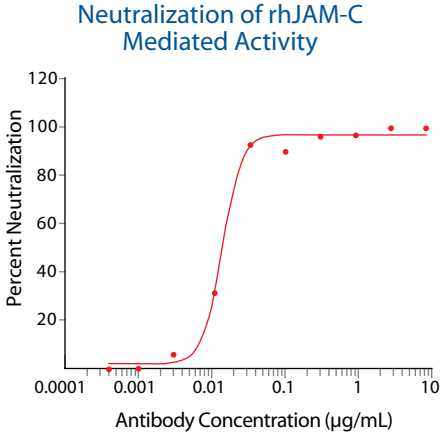


Figure 1. R&D Systems anti-human JAM-C monoclonal antibody (Catalog # MAB1189) inhibits JAM-C-mediated adhesion of human T cell leukemia J45.01 cells to the substrate.

ANALYTE	ANTIBODIES	PROTEINS
Cadherin-4/R-Cadherin	H	
Cadherin-6		H
Cadherin-8		H
Cadherin-11	H	H
Cadherin-12	H	H
Cadherin-17	H	
E-Cadherin	H M	H M
N-Cadherin		H
P-Cadherin	H M	H M
VE-Cadherin	H M	H
CD44	H	
DC-SIGN	H	H
DC-SIGNR	H	H
DNAM-1	H	H
Endostatin	H M	
Integrin α2/CD49b	H M	
Integrin α3/CD49c	H M	H
Integrin α3β1/VLA-3		H
Integrin α4/CD49d	H M	
Integrin α5/CD49e	H M	H
Integrin α6/CD49f	H M B	
Integrin αE/CD103	M	
Integrin αM/CD11b	H M	
Integrin αV/CD51	H	
Integrin αVβ3	H	
Integrin αVβ5	H	
Integrin αX/CD11c	H	
Integrin β1/CD29	H M	H
Integrin β2/CD18	H M	
Integrin β3/CD61	H	
Integrin β5	H	
Integrin β6	M	
Integrin β7	M	
JAM-A	H M	H M
JAM-B/VE-JAM	H M	H M
JAM-C	H M	H M
Laminin-1	M	
Laminin-5	H	
Netrin-1	M Ch	M Ch
Netrin-2	Ch	Ch
Netrin-4	H M	H M
Netrin-G1a	M	M
Netrin-G2a	M	
Nidogen-1	H	H
Porimin	H	
SPARC	H M	
SPARC-like 1	H M	

Key: **B** Bovine **Ch** Chicken **H** Human **M** Mouse

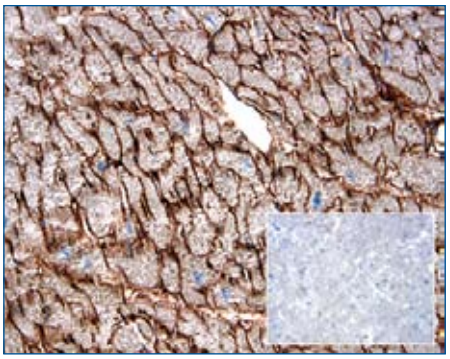


Figure 2. Detection of Nidogen-1 in paraffin-embedded human heart tissue sections using R&D Systems goat anti-human Nidogen-1 affinity purified polyclonal antibody (Catalog # AF2570). Tissues were stained using R&D Systems anti-goat HRP-DAB Cell and Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Inset shows control staining without primary antibody.

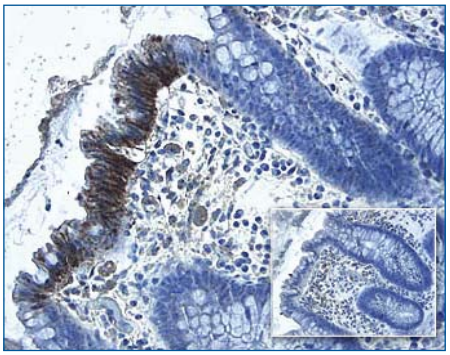


Figure 3. Detection of E-Cadherin in paraffin-embedded human colon tissue sections using R&D Systems mouse anti-human E-Cadherin monoclonal antibody (Catalog # MAB1838). Tissues were stained using R&D Systems anti-mouse HRP-DAB Cell and Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Inset shows control staining without primary antibody.

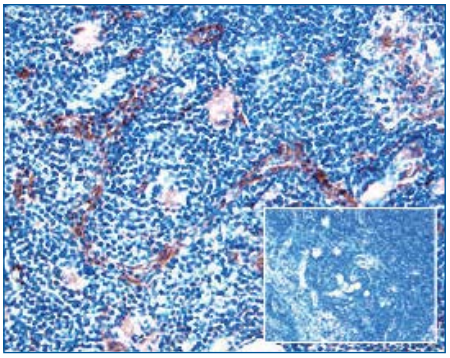


Figure 4. Detection of DC-SIGN/DC-SIGNR in paraffin-embedded human lymph node tissue sections using R&D Systems mouse anti-human DC SIGN and DC-SIGNR monoclonal antibody (Catalog # MAB1621). Tissues were stained using R&D Systems anti-mouse HRP-DAB Cell and Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Inset shows control staining without primary antibody.

Hedgehog, Wnt, & Notch Families

Secreted and membrane-bound signaling molecules of the Hedgehog, Wnt, and Notch families are well known for their roles in regulating tissue patterning and cell fate decisions during embryonic development. More recently, these molecules and their respective signaling pathways have been shown to function in the control of stem cell populations in adult tissues. Dysregulation of signaling in these populations frequently leads to malignant transformation.

Specifically, Hedgehog signaling has been implicated in cancers of the skin (basal cell carcinoma, BCC), cerebellum (medulloblastoma), and lung (small cell lung carcinoma, SCLC), among others. Hedgehog ligands, receptors (Patched and Smoothened), and transcriptional mediators (Gli) all appear to be targets for oncogenic mutations. In the canonical Wnt pathway, Wnt ligands signal through receptor complexes containing Frizzled and LRP 5/6. This impacts intracellular components axin, disheveled, GSK-3β and APC, which function to regulate the activity and subcellular localization of β-Catenin. Alternative Wnt pathways, the Wnt/Ca^{2+} and planar cell polarity pathways, have also been characterized. Dysregulated Wnt signaling is implicated in colorectal cancer and leukemias of both myeloid and lymphoid lineages. Notch pathway components have been implicated in a subset of acute lymphoblastic leukemias, as well as SCLC and BCC. Interaction of Notch receptors with ligands Delta-like or Jagged leads to proteolytic cleavage and release of the active intracellular domain of Notch (ICN), which translocates to the nucleus where it interacts with transcriptional regulators.

Wnt-Related

ANALYTE	ANTIBODIES	PROTEINS
β-Catenin	H M R X	H
Dkk-1	H M	H M
Dkk-2	M	
Dkk-3	H M	
Dkk-4	H M	H
Frizzled-1	H M	M
Frizzled-2	M	M
Frizzled-3	H M	
Frizzled-4	H M	M
Frizzled-5	H	H
Frizzled-6	H M	
Frizzled-7	H M	M
Frizzled-8	M	M
Frizzled-9	M	
sFRP-1	H	H
sFRP-2		M
sFRP-3	H M	H M
sFRP-4	H	H
Glypican 2	H M	H M
Glypican 3	H	H
Glypican 5	H M	H M
Glypican 6	H M	
GSK-3	H M R	
ICAT	H	
Kremen-1	H M	M
Kremen-2	H M	H M
LRP-1	H	
LRP-6	H M	H M
MFRP	H	
Norrin	H	H
ROR1	H	
ROR2	H	
Soggy-1	H M	
WIF-1	H M	H
WISP-1/CCN4	H M	

ANALYTE	ANTIBODIES	PROTEINS
Wnt-1	M	
Wnt-3a	M	M
Wnt-4	M	
Wnt-5a	M	M
Wnt-7a	H	
Wnt-8a	M	
Wnt-10b	M	
Wnt-11	M	

Hedgehog

Desert Hedgehog	M	
Indian Hedgehog	M	M
Sonic Hedgehog	H M	H M
Gas 1	H M	
Hip	M	M

DSL Notch

TACE/ADAM 17	H	H M
DLL 1	H	
DLL 4	M	H M
DNER	M	
Jagged 1	H R	R
Jagged 2	H	
Notch-1	R	R
Notch-2	R	R
Notch-3	H M	H M

ELISAs/ASSAYS	SPECIES
β-Catenin	H
Dkk-1	H M
GSK-3	H M R
Sonic Hedgehog	M
TACE/ADAM 17	H
γ-Secretase	Ms

Key: H Human M Mouse Ms Multi-species R Rat X *Xenopus*

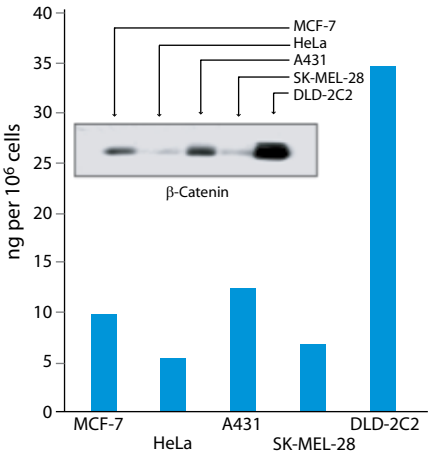


Figure 1. The amount of β-Catenin present in lysates prepared from human MCF-7, HeLa, A431, SK-MEL-28, and DLD-2C2 cells was quantified using R&D Systems Human Total β-Catenin DuoSet® IC ELISA (Catalog # DYC1329). The same lysates were also immunoblotted (inset) with R&D Systems anti-β-Catenin monoclonal antibody (Catalog # MAB1329). The DuoSet IC ELISA results correlate well with the total amounts of β-Catenin protein detected by Western blot.

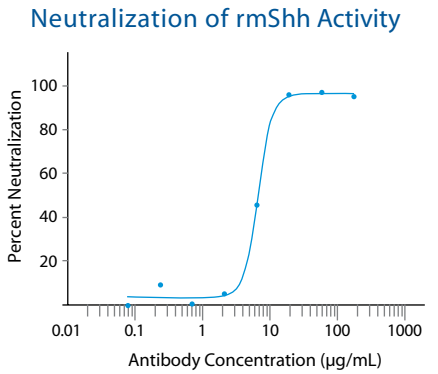


Figure 2. Recombinant mouse Shh protein (R&D Systems Catalog # 461-SH-025) induces alkaline phosphatase production by C3H10T1/2 fibroblasts (not shown). This activity is blocked by addition of R&D Systems anti-human/mouse Shh N-terminal peptide monoclonal antibody (Catalog # MAB4641). This antibody also blocks the activity of rhShh protein.

The CXCL12/CXCR4 Axis in Human Cancer

An increasing number of studies reveal a role for the chemokine receptor CXCR4 and its ligand CXCL12/SDF-1 in the progression and spread of a variety of tumors. Non-hematopoietic tumors including breast, colorectal, melanoma, osteosarcoma, ovarian, pancreatic, prostate, and small cell lung cancer have all been shown to express CXCR4 and/or respond to CXCL12. The expression of CXCR4 in some hematologic malignancies is not surprising in light of the expression of CXCR4 on subsets of normal peripheral blood lymphocytes. However, the level of CXCR4 expression can have prognostic value in some leukemias. CXCR4 exists in antigenically distinct conformations on many cell lines and primary cell types. Antibodies are available that recognize various epitopes of CXCR4, whose expression may or may not be evident on select tumor cell types.

Produced primarily by stromal cells, CXCL12 serves as a chemoattractant for CXCR4 bearing T cells, monocytes, and tumor cells. CXCL12 also exhibits angiogenic activity. Accordingly, a role for CXCL12 in promoting tumor cell migration and metastasis has been established in some tumor models. Interestingly, malignant plasma cells in multiple myeloma also secrete CXCL12/SDF-1. Strategies aimed at blocking CXCL12/CXCR4 interactions, originally developed for HIV therapy, may also be useful in the treatment of cancer.

ANALYTE	ANTIBODIES	ELISAs/ASSAYS	FLOW CYTOMETRY KITS	mRNA QUANTIFICATION KITS	PROTEINS
CXCR4	H M			H	
CXCL12/SDF-1	H M	H M	H	H	H M F RM

Key: H Human F Feline M Mouse RM Rhesus/Macaque

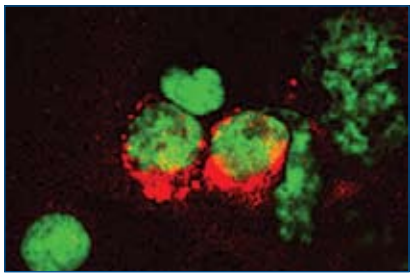


Figure 1. Detection of CXCR4 in human peripheral blood mononuclear cells using R&D Systems anti-human CXCR4 monoclonal antibody (Catalog # MAB172) and a rhodamine red X-conjugated anti-mouse secondary antibody. Cells are counterstained with Fluoro Nissl Green.

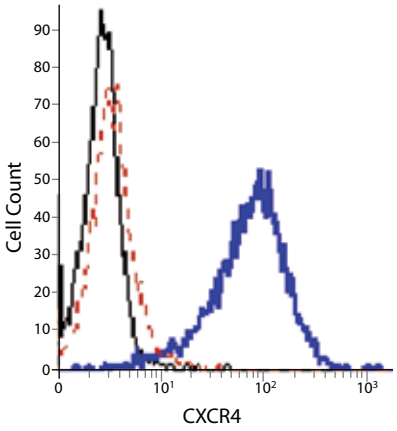
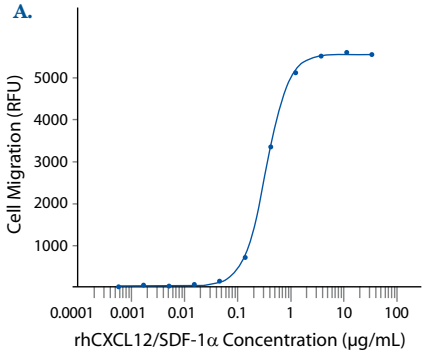


Figure 2. Quantitation of CXCR4 levels on the surface of Jurkat cells using the R&D Systems biotinylated SDF-1α Fluorokine® Kit (Catalog # NNS00). FACS analysis is shown for cells incubated with the biotinylated SDF-1α reagent (purple line), biotinylated SDF-1α plus blocking antibody (dotted red line - demonstrates specificity), and biotinylated negative control protein (thin black line).

Chemotactic Effect of rhCXCL12/SDF-1α on hCXCR4-Transfected BaF/3 Cells



Inhibition of Human CXCR4-Mediated rhCXCL12/SDF-1α Activity

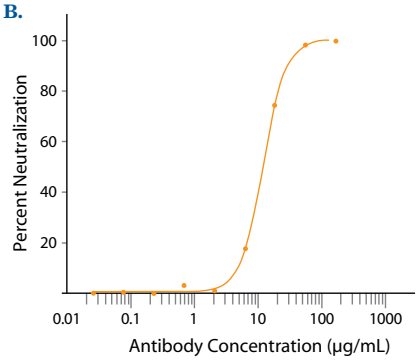


Figure 3. A. R&D Systems recombinant human CXCL12/SDF-1α (Catalog # 350-NS), exerts a chemotactic effect on BaF/3 cells that have been transfected with human CXCR4. The number of cells migrating is quantitated using R&D Systems Resazurin (Catalog # AR002) fluorescence. **B.** The chemotactic effect shown in **A** is blocked by the addition of increasing amounts of R&D Systems anti-human CXCR4 monoclonal antibody (Catalog # MAB173).

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