Thursday, 23 October 2008 08:00–09:45

PLENARY SESSION 4

Targeting autophagic pathways

239 INVITED

Oncogenes and tumor suppressor genes control autophagy

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Multiple oncogenes (in particular phosphatidylinositol 3-kinase, PI3K; activated Akt1; anti-apoptotic proteins from the Bcl-2 family) inhibit autophagy. Similarly, several tumor suppressor proteins (such as BH3-only proteins; death associated protein kinase-1, DAPK1; the phosphatase that antagonizes PI3K, PTEN; tuberous sclerosic complex 1 and 2, TSC1, TSC2; as well as LKB1/STK11) induce autophagy, meaning that their loss reduces autophagy. Beclin-1, which is required for autophagy induction acts as a haploinsufficient tumor suppressor protein, and other essential autophagy mediators (such as Atg4c, UVRAG and Bif-1) are bona fide oncosuppressors. One of the central tumor suppressor proteins, p53 exerts an ambiguous function in the regulation of autophagy. Within the nucleus, p53 can act as an autophagy-inducing transcription factor. Within the cytoplasm, p53 exerts a tonic autophagy-inhibitory function, and its degradation is actually required for the induction of autophagy. The role of autophagy in oncogenesis and anti-cancer therapy is contradictory. Chronic suppression of autophagy may stimulate oncogenesis. However, once a tumor is formed, autophagy inhibition may be a therapeutic goal for radiosensitization and chemosensitization. Altogether, the current state-of-the art suggests a complex relationship between cancer and deregulated autophagy that must be disentangled by further in-depth investigation.

240 INVITED

Role of autophagy in cancer resistance

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Drug resistance is a major obstacle that limits the effectiveness of cancer therapy. Therefore, we need to explore new strategies that overcome the emerging problem of drug resistance. Autophagy, an evolutionarily conserved response to stress, has recently been implicated in cancer initiation and progression. Accumulating evidence indicates that numerous cancer treatments cause autophagy, which is recognized as one of the resistance mechanisms against cancer treatment. In contrast, autophagy has also been thought to induce cell death by various cancer therapies, which is designated as type-2 programmed cell death or autophagic cell death. In tumor cells, the role of autophagy may depend on types of tumors or stimuli, stages of tumorigenesis, or extent of insult. Thus, it is essential to determine the role of autophagy in tumor cells in order to increase efficacy of the treatment by manipulating autophagic process. Today we present that a new alkylating agent temozolomide (TMZ) and γ-irradiation (IR), which are representative treatments for malignant glioma but not very effective, induce autophagy in vitro and in vivo settings and that inhibition of autophagy pharmacologically or genetically results in sensitization of tumor cells to TMZ and IR. Our data suggest that, when autophagy is cancer resistant mechanism, agents that disrupt autophagy are a promising new strategy to enhance the efficacy of cancer therapies in drug resistance.

241 INVITED

Novel therapeutic targets within the autophagic pathway

W.N. Hait. *USA*

Abstract not received

242 INVITED

Targeting apoptosis-resistant cancer cells through autophagic cell death

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Background: Lung cancer remains the leading cause of cancer death worldwide. Radioresistance of lung cancer cells results in unacceptable rate of loco-regional failure. Although radiation is known to induce apoptosis, our recent study showed that knockdown of pro-apoptotic proteins Bak and Bax resulted in an increase in autophagic cell death and lung cancer radiosensitivity in vitro. To further explore the potential of apoptosis inhibition as a way to sensitize lung cancer for therapy, we tested M867, a novel chemical and reversible caspase-3 inhibitor, in combination with ionizing radiation in vivo and in vitro.
Methods and Findings: M867 reduced clonogenic survival in H460 lung cancer cells (DER = 1.27, p = 0.007) compared to the vehicle-treated treated cells. We found that administration of M867 with ionizing radiation in an in vivo mouse hind limb lung cancer model was well tolerated, and produced a significant tumor growth delay compared to radiation alone. A dramatic decrease in tumor vasculature was observed with M867 and radiation using von Willebrand factor staining. In addition, Ki67 index showed >5-fold reduction of tumor proliferation in the combination therapy group, despite the reduced levels of apoptosis observed with terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining. Radiosensitizing effect of M867 through inhibiting caspases was validated using caspase-3/-7 double-knockout (DKO) mouse embryonic fibroblasts (MEF) cell model. Consistent with our previous study, autophagy contributed to the mechanism of increased cell death, following inhibition of apoptosis. In addition, matrigel assay showed a decrease in in vitro endothelial tubule formation during the M867/radiation combination treatment.
Conclusions: M867 enhances the cytotoxic effects of radiation on lung cancer and its vasculature both in vitro and in vivo. M867 has the potential to prolong tumor growth delay by inhibiting tumor proliferation. Clinical trials are needed to determine the potential of this combination therapy in patients with locally advanced lung cancer.

Thursday, 23 October 2008 10:15–12:00

PLENARY SESSION 5

Molecular targets – state of the science C

243 INVITED

Aurora kinase inhibitors: more than one opportunity?

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Aurora serine/threonine kinases are a family of three proteins, which play key roles in critical phases in mitosis in controlling chromosome assembly and segregation. Inhibition of Aurora A (AA) function results in cell cycle arrest and monopolar mitotic spindles formation. Inhibition of Aurora B (AB) results in a premature exit from mitosis due to abolishment of a critical spindle checkpoint and in endoreduplication. Aurora C (AC) has been less studied. The interest for Aurora kinases inhibition as an anticancer strategy arose from the hope to target mitosis more specifically and from the over expression of Aurora kinases in most human cancers where AA gene is frequently amplified. Ectopic over expression of AA was shown to induce oncogenic transformation, while ablation resulted in cell death in tumor cells but in reversible cell cycle arrest in normal cell. Interestingly, AA over expression was shown to induce resistance to some chemotherapeutic agents through different mechanisms. Although an oncogenic activity of AB was not demonstrated, elevated AB activity is known to promote Ras-mediated transformation by enhancing oncogenic signaling and by inducing aneuploidy. It was also observed that patients with AB-positive carcinoma had a poor prognosis compared to those with AB negative tumors. The link of AC and cancer is less well defined.
Initially a few companies started to work on small molecule Aurora inhibitors and the first 2 phase I clinical studies have been initiated in solid tumors in June 2004 by Nerviano Medical Sciences with PHA-739358. To date more than 20 programs are on going in various companies, and more than 10 compounds have reached the stage of clinical trials. These compounds are selective Aurora kinase A or B inhibitors, or pan-Aurora kinase inhibitors. Most compounds show some cross-reactivity with other cancer-relevant kinases including Abl (wt and T315I), Ret, TrkA, FGFRs, VEGFRs, JAK2, or FLT3.
In solid tumors dose limiting toxicities were essentially neutropenic infection and febrile neutropenia. Some specific compounds were put on hold due to CNS events, prolongation of QTc, or pulmonary issues. Safety profiles consist mainly of grade (G) 3–4 neutropenia, G1–2 fatigue, anorexia, and nausea. G1–2 diarrhea in up to 50% of patients is mainly seen in the case of oral inhibitors. Reduction of histone H3 phosphorylation has been described as a pharmacodynamic biomarker of AB inhibition in human tissues. Clinically relevant stable diseases have been described in most publications in various tumors. Preliminary clinical efficacy starts to be reported in various tumor types: partial responses in ovarian cancers in single agent trials at recommended phases II doses, and in small cell lung cancer with G-CSF support. Better characteristics of patients who might benefit from Aurora inhibition have still to be elucidated.

Hematological malignancies:
- Publications on 3 drugs, which are pan-Aurora inhibitors targeting also Bcr-Abl kinase, described hematological responses in early clinical trials in patients with T315I or other Bcr-Abl mutated CML (and Philadelphia positive ALL in 2 cases) relapsing on imatinib and other c-Abl therapies. Partial and complete cytogenetic responses were reported, with undetectable T315I in one case.
- In AML, reduction of bone marrow blasts was observed. This preliminary antileukemic activity was not sustained enough and led to investigate new regimens with dose-intensifications and more prolonged administrations. Similarly more frequent administrations/intakes of drugs targeting also FLT3 aim at maintaining a sufficient inhibition for a prolonged diminution of blasts.
- Myeloproliferative disorders: cross-reactivity with JAK2 was explored with one compound and is expected to be investigated for other Aurora inhibitors.

Potential other targets: The different cancer relevant cross-reactivities of some compounds might be at the origin of specific developments, such as inhibition of FGFRs for example in T(4−14) positive Multiple Myeloma, or in subsets of patients with bladder, breast, endometrial and cervix cancers, or Ret in thyroid cancers.
Combinations: Preclinical studies support a wide spectrum of combinations with classical chemotherapeutic agents or targeted drugs. Phase I combination studies are ongoing and might represent the most promising future of Aurora kinase inhibitors.
Updated results on these compounds will be given during the lecture.

244 INVITED

Polo-like kinase inhibition in oncology: from bench to bedside

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Background: *polo* is a gene encoding for an enzyme present in *Drosophila melanogaster*, discovered and cloned in 1991 by Llamazares *et al.* Mutations in *polo* were found to induce abnormal mitoses in *Drosophila*. Clay *et al.* identified and cloned gene sequences in murine hematopoietic progenitor cells encoding for a protein kinase, which shares extensive homology with the enzyme encoded by *Drosophila*'s *polo* gene. The mouse gene was named *Plk*, the protein encoded by *Plk* is polo-like kinase, which belongs to a family of serine/threonine kinases with critical involvement in cell growth and differentiation in various species. Hamanaka *et al.* cloned in 1994 both murine and human complementary DNAs that were homologous to *Drosophila*'s *PlkIT*> gene and the related kinase. The human counterpart of the mouse gene was named P*LK*. Up to 80% of human tumors of various origin express high levels of *PLK* transcripts, whereas the according mRNA is mainly absent from surrounding healthy tissue, as described first by Holtrich *et al.* in 1994. Polo-like kinase expression is associated with poor prognosis in some tumor types.
Materials and Methods: Polo-like kinases 1, 2 and 3 are key regulators of multiple steps in mitosis and obviously an attractive target for anticancer drug development. The current state-of-the-science presentation will briefly describe the biology of polo-like kinases and the basic principles of inhibiting these enzymes. Polo-like kinase inhibitors interfere with different stages of mitosis, with centrosome maturation, spindle formation, chromosome separation and cytokinesis. They induce mitotic chaos and severe pertubation of the cell cycle, ultimately leading to cancer cell death. Various drugs in advanced stages of preclinical and early clinical testing will be reviewed. Non-confidential safety and efficacy data from the first dose-finding Phase I studies and the earliest screening Phase II trials with polo-like kinase inhibitors will be presented. The pharmacology of the most advanced agents will be summarized briefly. The presentation will cover information on drugs such as BI-2536, BI-6727, GSK461364, LC-445, various pyrazoloquininazolines, ON 01910, amongst others.
Conclusions: Small-molecule inhibitors of polo-like kinases are new and promising tools in the treatment of human cancers, some of them having passed the transition from bench to bedside and now reaching the critical Phase I/early Phase II stage of clinical development in solid tumors, lymphoma and hematological malignancies.

245 INVITED

PARP inhibitors in cancer treatment

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Poly(ADP-ribose) polymerases (PARPs) are a family of highly conserved enzymes, the most abundant being PARP-1, a nuclear DNA-binding enzyme that is activated by DNA strand breaks. PARP-1 has a key role in binding to and the signalling of DNA single strand breaks as part of the base excision repair process.
Recent evidence shows that BRCA-1 or BRCA-2 defective cell lines are exquisitely sensitive to PARP inhibition compared to BRCA wild-type or heterozygous cell lines [1,2]. This is because single strand breaks accumulate following PARP inhibition, which result in stalled replication forks and DNA double strand breaks during S-phase. These lesions are normally repaired by homologous recombination repair, which is dependent on BRCA functionality. BRCA functionality may be lost in a significant proportion of cancers by epigenetic mechanisms as well as by hereditary mutations, increasing the population potentially sensitive to treatment with PARP inhibitors.
AG014699, a potent tricyclic indole inhibitor of PARP-1 and 2, was the first in class to undergo a Phase I trial in cancer patients [3]. AZD2281 (KU-0059436) has undergone a Phase I trial in cancer patients with BRCA mutations [4]. Phase I data have been reported on BSI-201 and Phase 0 data on ABT-888. A Phase II study of AG014699 in patients with breast or ovarian cancer and known mutations of BRCA-1 or BRCA-2 is being conducted in UK centres.

References

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[4] Peter C Fong et al, AZD2281 (KU-0059436), a PARP (poly ADP-ribose polymerase) inhibitor with single agent anticancer activity in patients with BRCA deficient ovarian cancer: Results from a phase I study. Proc ASCO 2008, Abstract No: 5510.

246 INVITED

Targeting Her: Can resistance to EGFR inhibitors be overcome?

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Background: The epidermal growth factor receptor (Her1) is a receptor tyrosine kinase with an extensively described role in mediating pleiotropic cellular responses. Upon binding by a number of ligands, the receptor enters into ligand-receptor dimers and is autophosphorylated. The phosphorylated receptor recruits docking proteins and signal transduction molecules, resulting in activation of cascades which are anti-apoptotic, proliferative, and contribute to metastasis and angiogenesis. Targeting of Her is therapeutic in lung, head and neck and colon cancer, but de novo and acquired resistance are common, attributed to mutation in downstream effectors, upregulation of redundant receptors, mutation in the receptor, and alteration in receptor trafficking, among other mechanisms.
Materials and Methods: A systems biology approach defined an siRNA library targeting the extended EGFR signaling network. High throughput screening of this library identified candidate genes that sensitize cells to EGFR inhibition. This dataset is used to map elements of the EGFR signaling network responsible for sensitivity.
Results: 110 primary hits sensitized to one or both of the EGFR-targeting agents, with significant overlap within the groups. Sensitization effects are confirmed with 4 separate siRNAs targeting each hit, establishing that the degree of sensitization correlates with potency of the siRNA in knocking down expression of its target. Approximately 45% of the initial hits pass validation. A number of the hits are quite distant from EGFR in the protein interaction networks, including novel candidates for regulating the EGFR pathway.
Conclusions: siRNA targeting of a library of candidate genes identifies novel targets for sensitization to EGFR inhibition, and provides a tool for drug discovery and design of trials for patients with cetuximab or erlotinib resistant head and neck, colon and lung cancer.