Review

Molecular basis of targeted chemotherapy: Novel concepts with special reference to the treatment of Hodgkin’s disease

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Summary
Concepts for the treatment of Hodgkin’s lymphomas based on novel insights of the molecular mechanisms responsible for the maintenance of the transformed phenotype of Reed-Sternberg cells, their proliferation and sensitivity to radiation and antitumor agents are discussed. The potentials of some recently developed new signal transduction inhibitors for the treatment of Hodgkin’s lymphomas are discussed in greater detail and comprise agents directed against Janus kinase 2 (JAK 2); Signal Transducers and Activators of Transcription (STAT factors); agents directed against SH 2-domains; the fes/fps oncogene, Ras; protein kinase C (PKC) isotypes and means of inducing radiation or drug-induced apoptosis.

Key words: bcl-2, fes/fps, JAK, protein kinase C, Raf-1, Ras, STAT

Introduction
The concept of employing elements of mitogenic signal transduction as targets for cancer chemotherapy originated from expanding knowledge of the mechanism of transformation by oncogenes. It has become clear that most oncogene products can be classified into three categories: (i) growth factors; (ii) growth factor receptors; or (iii) elements of growth factor signal transduction, including transcription factors. All these molecules are part of the information-transfer system regulating cellular proliferation. Malignant transformation can be described as a dysfunction of signal transduction, resulting in autonomous growth where cells either generate their own growth-promoting stimuli or do not respond to growth-inhibitory signals. Agents which are able to counteract oncogene-induced growth stimulation may potentially act as tumor-specific drugs. They could act by interference with autocrine cycles, by blocking constitutively active growth factor signal transduction elements, or by restoring the function of deficient suppressor gene products. Present activities along these lines include, in particular, development of growth factor antagonists, growth factor receptor blockers and targets in growth factor signal transduction at the post-receptor level [1, 13].

The treatment of Hodgkin’s lymphomas has developed into a success story in clinical oncology. However, despite tremendous progress, there is still a significant percentage of treatment failures or patients with relapse refractory to further treatment. Although the percentage of these cases may be low compared to other malignancies, it remains a challenge. Obviously, novel strategies are required in order to cope with these situations.

In the following sections possible new chemotherapeutic strategies will be discussed based on recent insights into the molecular basis of growth control and response to antitumor agents.

Novel targets
The compounds which are presently employed in the chemotherapy of Hodgkin’s lymphomas were developed at a time when the knowledge of the molecular mechanisms governing cellular proliferation of normal and malignant cells was rather limited. In the meantime, a wealth of data has accumulated in this area revealing a long list of attractive new targets for pharmacological intervention in tumor growth. Table 1 lists some of the presently addressed targets in mitogenic signal transduction.

Although the origin of Hodgkin cells is still a matter of debate, there is increasing evidence that Hodgkin or Reed-Sternberg cells arise from a B-cell lineage (see contributions to this meeting). Thus, if we look for new targets for the treatment of Hodgkin’s lymphomas, mechanisms involved in the regulation of cellular proliferation of hematopoietic cells are of primary interest. Furthermore, Hodgkin cells have been reported to secrete a number of cytokines comprising IL-2, IL-1, IL-6, IL-9, TGF-β and the corresponding receptors [2, 3]. In addition to these potential autocrine cycles, Hodgkin cells may be stimulated in a paracrine fashion by neighboring lymphocytes [3].

Considering this background, are there any specific elements of the signaling cascade from hematopoietic...
Table 1. Targets of signal transduction inhibitors (selection).

<table>
<thead>
<tr>
<th>Target</th>
<th>Inhibitor</th>
</tr>
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<tbody>
<tr>
<td>VEG/Flik-1 (KDR)</td>
<td>PDGF/PDGFR</td>
</tr>
<tr>
<td>IGF-1/IGF-1R</td>
<td>EGFR/EGFR</td>
</tr>
<tr>
<td>pp60^Src</td>
<td>JAK-2</td>
</tr>
<tr>
<td>Phosphatases</td>
<td>STAT factors</td>
</tr>
<tr>
<td>Ras farnesyltransferase</td>
<td>Fps/fes</td>
</tr>
<tr>
<td>She/Grb2</td>
<td>FGF/FGFR^c</td>
</tr>
<tr>
<td>SH2 domains</td>
<td>CSF/CSF-1R</td>
</tr>
<tr>
<td>SH3 domain</td>
<td>Bcr-Abl</td>
</tr>
<tr>
<td>Raf-1</td>
<td>NFKB</td>
</tr>
<tr>
<td>MAPK/Mek/Mekk</td>
<td>PKC isoenzymes</td>
</tr>
<tr>
<td>Cdk2/Cdc2/cdks 4-6</td>
<td></td>
</tr>
<tr>
<td>Transcription factors: Myc, Fos, p53</td>
<td></td>
</tr>
</tbody>
</table>

^a VEGF, vascular endothelial growth factor; Flik-1 (KDR), VEGF receptor.
^b IGF-1, insulin-like growth factor-1; IGF-1R, IGF-1 receptor.
^c FGF, fibroblast growth factor; FGF FR, FGF receptor.
^d SH2, Src homology 2.
^e CSF, colony-stimulating factor; CSF-1R, CSF-1 receptor.
^f SH3, Src homology 3.
^g MEK, MAPK kinase; MEKK, MAPK kinase.
^h STAT signal transducers and activators of transcription.

cytokine receptors which represent potentially useful targets for novel agents in the chemotherapy of Hodgkin's disease? Some candidate targets which should be considered under this aspect are marked in Table 1. This list of attractive new targets includes Janus kinases, STAT factors, proteins with SH2-domains, Ras, p53, and some protein kinase C isoenzymes.

Janus kinases

Most hematopoietic cytokines employ non-receptor tyrosine kinases which associate with the activated receptor complex. Erythropoietin, granulocyte/macrophage colony stimulating factor (CSF), thrombopoietin, and the interleukins (IL) 3, 4, 5, 7, 8, 10, and 16 signal through Janus kinases (JAKs). These enzymes contain two catalytic tyrosine kinase domains, of which only one appears to be functionally active. These two catalytic sites resembling the double-faced Roman god Janus, lead to their designation as Janus kinases or JAK. The family of JAKs has been divided into four groups: JAK1, JAK2, JAK3, and TYK2. The various cytokine receptors differ with regard to the JAK type employed [4]. Obviously, the JAK family represents the most attractive targets for new antitumor agents directed against malignancies of the hematopoietic system. Recently, the structure of a JAK2-specific inhibitor has been published [5]. JAK2 is the main growth driver for leukemic pre B cells. The JAK2 inhibitor AG490 proved to be a potent non-toxic inhibitor of human pre B-lymphoblastic leukemia cells grown in SCID mice [5]. Since B-cell markers are expressed on Hodgkin cells, this JAK2 inhibitor may also conceivably affect the growth of Hodgkin lymphomas. In any case, it appears rewarding to look for selective inhibitors targeted to other members of the JAK family. Preparation of highly selective tyrosine kinase inhibitors has been most successful. Synthesis of JAK1, -3, or TYK2-specific agents should therefore be initiated.

STAT factors

JAKs transmit signals to cytokine-responsive genes by STAT factors, where STAT represents an acronym for signal transducers and activators of transcription. STAT factors represent a family of structurally closely related proteins encoded in mammals by seven different genes. STAT factors bind to the ligand-activated cytokine receptor complex through their SH2 domains which dock to phosphorylated tyrosyl residues of the receptor [4]. Recruitment of the STAT factor to the activated receptor permits a phosphorylation of STAT by the receptor-associated JAK. Tyrosine phosphorylation of STATs triggers their dimerization and translocation to the nucleus where they bind to specific DNA sequences. These STAT factors represent additional attractive targets for pharmacological intervention. Specific STAT inhibitor proteins have been described [6, 7]. These proteins could be expressed in Hodgkin cells by targeted vectors or their action mimicked by peptidomimetics.

SH2 blocking agents

Whereas this may be a task for the future, an alternative approach is currently pursued, i.e. synthesis of selective SH2 blocking agents [8]. The development of compounds interfering with SH2 domain protein interaction either by interacting directly with the SH2 domain or by masking the corresponding phosphorylated tyrosyl residue has become a hotspot in anti-cancer drug design [8]. Interesting new compounds also with regard to Hodgkin’s disease are to be expected from these efforts.

Fes/fps oncogene

In addition to JAKs, hematopoietic cells – especially lymphocytes – employ a number of additional non-receptor tyrosine kinases like Fyn, Lck, Zap, Src, and others for mitogenic signaling [9, 10]. A major role of these nonreceptor tyrosine kinases is the activation of the Ras–Raf-1–MAP kinase pathway which has to cooperate with the JAK-STAT signaling for growth stimulation. In one carefully conducted study, 75% of all investigated Hodgkin cells expressed the fes/fps oncogene [11]. This oncogene encodes a nonreceptor tyrosine kinase which resembles the Src-related kinases as well as Syk and Zap by containing an SH2 domain, suggesting that they may dock to phosphorylated tyrosyl residues. Although the biological function of Fes/Fps is presently unknown, the fact that they exhibit transforming potency in animal systems suggest an important role of these tyrosine kinases in mitogenic signaling and possibly in...
the pathogenesis of Hodgkin's disease. Fps has recently been shown to be the preferred target of the Bcr/abl protein in CML cells, emphasizing the possible relevance of this kinase for malignant transformation [12]. Inhibitors interfering with nonreceptor tyrosine kinases are available [13]. These inhibitors may provide lead substances for the synthesis of fes/fps-specific inhibitors, which should be investigated with regard to their ability to suppress the proliferation of Hodgkin cells.

Ras

As outlined above, activation of the Ras–Raf-1 pathway appears to be essential for growth stimulation of most cell types including those derived from hematopoietic lineages [14]. The essential role of Ras in mitogenic signaling together with the fact that mutated Ras is one of the most frequent oncogenes expressed in human tumors has made Ras a particularly attractive target for novel antitumor agents. The inhibition of Ras farnesylation has proven to be the most successful strategy so far. Interestingly, these farnesyltransferase inhibitors turned out to be relatively nontoxic [15]. This may be due to the fact that only H-Ras is inhibited by farnesyltransferase inhibitors (FTI) [15]. In presence of FTIs, the Ras iso-
types K- and N-Ras can be activated by geranylgeranylation and compensate the blockade of H-Ras [15–17]. This raises the possibility that in cases where tumor growth is not under the control of an activated H-Ras – which is indeed relatively rare – Ras may not be the essential target of the FTIs. The Ras homologue Rho is another protein which requires farnesylation for functional activity. Inhibition of Rho reverses transformation by Ras [18, 19]. Thus, inhibition of RhoB farnesylation may be responsible for the antitumor effect of FTIs [16]. In any case, FTIs should be studied with regard to their effect on Hodgkin's disease.

Protein kinase C

It has become clear that an activation of Rho is essential for transformation by oncogenic Ras [18, 19]. Rho is involved in the regulation of actin stress fiber formation [20, 21]. Stress fibers are anchored at the plasma membrane through special structures termed focal adhesion complexes [22]. They are essential for the organization of the signaling elements involved in integrin signaling [22]. We could demonstrate that atypical protein kinase C (PKC) \( \lambda \) is required for this pathway [23]. Expression of a dominant negative \( \lambda \) mutant reverses transformation by oncogenic Ras [24], emphasizing the biological significance of active PKC \( \lambda \) for the preservation of the transformed phenotype. Studies to prepare PKC \( \lambda \)-specific inhibitors have been initiated. For the reasons mentioned above, PKC \( \lambda \) may also represent a promising new target for the chemotherapy of Hodgkin's disease.

Inducers of apoptosis

Resistance to current chemotherapy protocols may also be due to a defective apoptotic pathway in the resistant Hodgkin cells. Inability to enter apoptosis is frequently associated with a deletion or mutation of p53. Expression of mutated p53 has indeed been reported to occur in Hodgkin cells with considerable frequency [11]. Strategies have been described to selectively kill cells over-expressing p53 or cells in which both p53 alleles are deleted [25]. These strategies, which are still in a very early experimental state, should be of special relevance for the treatment of resistant Hodgkin cells. Other concepts to overcome anti-apoptotic mechanisms which may be active in resistant Hodgkin cells include depletion of bcl-2 by antisense constructs [26], PKC \( \lambda \) inhibitors, and inhibition or depletion of Raf-1. The atypical PKC iso-
types \( \lambda/\lambda \) are of special interest, as they appear to act relatively far downstream in the apoptotic signaling cascade where several apoptotic pathways converge [27, 28]. Evidence has shown that they function downstream of bcl-2 and may be involved in the regulation of the caspases [28]. As the same isoforms are also essential for the mitogenic cascade, inhibitors of these PKC iso-
types would act in a dual fashion, i.e. inhibit growth and induce apoptosis. A very similar effect is to be expected from Raf-1 inhibition. Raf-1 is an essential element of mitogenic signaling but at the same time acts as a powerful anti-apoptotic agent [29]. Thus Raf-1 inhibitors have been suggested as novel, potentially potent antitumor agents, a model which is presently being evaluated in several laboratories.

In summary, the molecular biology of growth regulation in normal and transformed cells as well as the biochemical response mechanisms following exposure to radiation and antitumor drugs have identified an impressive list of novel targets for chemotherapeutic interventions. Considering the redundancy of most mitogenic signaling pathways, these agents should not, however, be employed in the form of monotherapy but principally in the form of 'intelligent combinations', thereby ensuring growth inhibition and minimizing the development of drug resistance.

References

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