Osteosarcoma : searching for new treatment options

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SUMMARY

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Osteosarcoma is the most frequent high-grade primary malignant bone tumor that is thought to arise from mesenchymal stem cells with the capacity to produce osteoid. The overall incidence is of three cases per million annually, and it occurs predominantly in children and adolescents as well as in people over 50 years of age.

Currently, the treatment consists of preoperative chemotherapy followed by resection of the tumor. The most effective systemic chemotherapeutics are cisplatin, doxorubicin and methotrexate. Despite extensive studies aimed at finding optimal combined chemotherapeutic strategies, overall 5-year survival rates have not increased above 70%, and around 35-45% of the patients have tumors that do not respond to chemotherapy.

The aim of this thesis was to discover new therapeutic options for osteosarcoma patients. I focused on finding candidate targets and pharmaceutical inhibitors for killing human osteosarcoma cells or for sensitizing osteosarcoma cells to doxorubicin. In **Chapter 2** I studied Aven, an adaptor protein that has been implicated in anti-apoptotic signaling and in DNA damage response signaling. The expression of Aven is inversely correlated with metastasis-free survival in osteosarcoma patients, and is increased in metastases compared to primary tumours. In tumour cells, silencing Aven triggered a G2 cell cycle arrest. Chk1 protein levels were attenuated and ATR-Chk1 DNA damage response signaling in response to chemotherapy was abolished in Aven-depleted osteosarcoma cells while ATM, Chk2, and p53 activation remained intact. It is not possible to target Aven, therefore I examined whether pharmacological inhibition of the Aven-controlled ATR-Chk1 response could sensitize osteosarcoma cells to doxorubicin. For this purpose, I tested pharmacological inhibitors targeting Chk1/Chk2 or selectively Chk1 in 2D and 3D cultures. Co-treatments in both culture systems led to effective sensitization to chemotherapy. Together, these findings implicate Aven in ATR-Chk1 signaling and point towards Chk1 inhibition as a strategy to sensitize human osteosarcomas to chemotherapy.

An siRNA screen targeting members of the Bcl-2 family in human osteosarcoma cell lines to identify critical regulators of osteosarcoma cell survival was performed in **chapter 3**. Silencing the anti-apoptotic family member Bcl-xL but also the pro-apoptotic member Bak caused loss of viability. Loss of Bak impaired cell cycle progression and triggered autophagy. Instead, silencing Bcl-xL induced apoptotic cell death. Clinical osteosarcoma samples showed expression of Bcl-xL, but mRNA or protein levels did not significantly correlate with therapy response or survival. Nevertheless, pharmacological inhibition of Bcl-xL synergistically

enhanced the response to the chemotherapeutic agent, doxorubicin. Indeed, in osteosarcoma cells strongly expressing Bcl-xL, the Bcl-xL-selective BH3 mimetic, WEHI-539 potently enhanced apoptosis in the presence of low doses of doxorubicin. Our results identify Bcl-xL as a candidate drug target for sensitization to chemotherapy in patients with osteosarcoma.

In **Chapter 4** I performed a kinase inhibitor screen in two osteosarcoma cell lines, which identified MEK1/2 inhibitors: Trametinib, AZD8330 and TAK-733. These inhibitors were further validated in a panel of six osteosarcoma cell lines of which three were sensitive and three resistant to these inhibitors. Western blot analysis revealed that sensitive lines had high constitutive ERK activity. Furthermore, experiments in which the cell lines were cultured in a 3D culture system and exposed to the inhibitors, validated the effect seen in 2D monolayer cultures. A gene expression analysis was performed to identify differentially expressed gene signatures in sensitive and resistant cell lines, and indicated an activation of the AKT signaling network in the resistant cell lines. In conclusion, MEK1/2 inhibition represents a candidate treatment strategy for osteosarcomas displaying high MEK activity as determined by ERK phosphorylation status.

Chapter 5, focuses on elucidating the effect of three Src inhibitors, dasatinib, bosutinib and saracatinib, on osteosarcoma viability and cell migration using 2D cultures and validation in 3D culture systems. Expression and activity of the Src cytoplasmic tyrosine kinase has been correlated with clinical stage and survival. All inhibitors were tested in combination with doxorubicin showing a reduction of the IC50 of this chemotherapeutic. However, only dasatinib treatment triggered caspase3/7 activation, and decreased Src activity. The effects of the inhibitors were studied in 3D extracellular matrix (ECM) scaffolds. Under these conditions, all three inhibitors reduced viability but formation of branched networks in 3D cultures was selectively inhibited by dasatinib in presence of doxorubicin. The activity of focal adhesion kinase (FAK), a Src substrate that is important for cell migration, was exclusively sensitive to dasatinib. Additionally, in 3D ECM-embedded spheroid cultures dasatinib blocked cell migration capacity whereas the other inhibitors had no or partial effects. Together, these findings point to the use of dasatinib as a candidate drug to enhance apoptosis in response to chemotherapy and to reduce metastatic spread in patients with osteosarcoma.

In summary, the work presented in this thesis provides four new candidate treatment options for osteosarcoma. These studies provide the basis to continue this research in animal models, which may then be translated to the clinic.