

Table of contents

1	List of figures	I
2	List of tables	II
3	List of abbreviations	III
4	Zusammenfassung	VI
5	Summary	VIII
6	Introduction	I
6.1	Mast cell maturation, characteristics, functions and pathologies	I
6.2	KIT signaling pathway	7
6.2.1	SM therapy with TKIs (Imatinib, Midostaurin)	9
6.3	IL-2-dependent signaling and role of IL-2R α in the context of mastocytosis ..	10
6.3.1	CD25 as a diagnostic marker	13
6.3.2	Impact of Ruxolitinib on the IL2 signaling pathway	15
6.3.3	STAT5 target genes	17
7	Aim of the dissertation	19
8	Materials and Methods	20
8.1	Materials	20
8.1.1	Chemicals	20
8.1.2	Cell culture media	22
8.1.2.1	Stimulation medium	24
8.1.3	Buffer and solutions	24
8.1.4	Laboratory KIT Systems	26
8.1.5	Antibodies, Inhibitor and Blocking solution for the experiments	27
8.1.6	Reverse transcription	28
8.1.7	Primer	28
8.1.8	Laboratory equipment and materials	30

8.2	Methods	31
8.2.1	Cell culture	31
8.2.2	Real-time quantitative PCR (RT-qPCR)	31
8.2.2.1	Cell preparation for qPCR	31
8.2.2.2	RNA isolation	32
8.2.2.3	Reverse transcription	32
8.2.2.4	RT-qPCR	33
8.2.3	Western Blot	34
8.2.3.1	IL-2 stimulation	34
8.2.3.2	Cell lysis	35
8.2.3.3	BCA protein assay	35
8.2.3.4	SDS-PAGE	36
8.2.3.5	Western Blot transfer	37
8.2.3.6	Immunodetection	37
8.2.3.7	Stripping protocol of WB membrane	38
8.2.4	FACS-Analysis	39
8.2.5	Statistics	40
9	Results	41
9.1	Analysis of CD25-expression in human mast cell lines	41
9.2	Fc Block improves specificity of CD25 detection	46
9.3	Identification of sub-populations in HMC-1.2	48
9.4	IL-2 receptor chains are differentially expressed in the HMC-cell lines	49
9.5	IL-2 activates CD25 signaling in HMC-1.2 cells	50
9.6	Ruxolitinib inhibits IL-2 mediated signaling in HMC-1.2 cells	52
9.7	CD25 signaling in HMC-1.1 vs. HMC-1.2 cells	54
9.7.1	IL-2 signaling mediated target gene expression in HMC-1.2 cells	55
10	Discussion	59
11	Outlook	64
12	References	A
