The Role of Ruthenium in Anti-Cancer Agents

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The role of transition metals in medicine is largely unexplored. One early exploration into this field occurred in 1931 when Collier and Krauss studied the effect of inorganic metal salts in mice implanted with Ehrlich's mouse carcinoma. Most of the effect that these compounds had seemed to be related to the ligands instead of the metal center, so the relevance of transition metal drugs died out.[1] Some 34 years Rosenberg reported that several Pt^{IV} halogenides, Rh^{III} chlorides, and the $[Ru^{III}(NH_3)_4Cl(OH)]Cl$ complex caused a filamentous growth pattern in *E. coli*. Consequently, these types of metals interfere with cell division and their effect was assumed to be caused by interaction with DNA.[2] Four years later, he showed *in vivo* inhibition of sarcoma and leukimia by platinum compounds, such as $PdCl_2(NH_3)_2$ (Cisplatin), and claimed these compounds as a new class of potent anti-tumor agents. A decade later, Pascoe showed that DNA is indeed the cellular target of cisplatin, and the anticancer drug was clinically approved in 1978.[3]

Ruthenium drugs were initially designed to mimic the mode of action of cisplatin, whereupon entering the body, the two Pd-Cl bonds would be hydrolyzed and later bond with the nitrogenated bases of the DNA to effectively block the replication process of the cell. The first effective ruthenium drugs to be designed were Ru^{III} prodrugs. These drugs were intended to go inside the body and be reduced into Ru^{II} by the hypoxic environment to tumor tissue. The first of these novel Ru^{III} drugs to be developed was done by Bernhard Keppler in 1986 when he introduced (H_2Im) *trans*- $[RuCl_4(HIm)_2]$ (KP418).[4] This drug was studied for its anticancer effect on autochthonous colorectal carcinoma in rats and showed above 90% inhibition of tumor growth, however this was accompanied by 26% loss of body weight and 10% mortality.[5] However, in this same study, it was found that the complex (H_2Ind) *trans*-$[RuCl_4(HInd)_2]$ (KP1029) reduced these side effects while retaining the antitumor activity. Both KP418 and KP1019 were shown to induce apoptosis *via* the mitochondrial pathway in colorectal carcinoma cell lines.[6] Then, by exchanging the indazolium counterion with sodium to form sodium *trans*-$[RuCl_4(HInd)_2]$ (IT-139) (**Figure 1**), a 30-fold increase in solubility was observed. IT-139 is now in phase-I/II clinical studies. [7,8] Treatment with IT-139 was shown to downregulate stress-mediated induction of GRP78. As upregulated GRP78 in cancer cells is necessary to enable metastatic growth in the lung microenvironment, IT-139 may be suitable to target metastatic progression in cancer patients. [9]

Alternative attempts to form Ru^{III} anticancer agents investigated the potential of S-donor ligands as an alternative to N-donor ligands. It was thought that the π-acceptor properties of these ligands would reduce the reduction potential of $Ru^{III} \rightarrow Ru^{II}$. In 1975, $[RuCl_2(DMSO)_4]$ was tested in *E. coli* and a filamentous growth, similar to that of when cisplatin was used, was observed.[10] In a systematic study, it was later found that the *trans*-isomer of this complex had better activity against a metastasizing Lewis lung carcinoma model than the *cis*-isomer.[11] This study also showed that *trans*-$[RuCl_2(DMSO)]$ only had marginal effect on the primary tumor but greatly reduced the volume of lung metastases. However, this species proved to be unstable in aqueous solution as it immediately released one DMSO ligand. After further optimization,

it was found that the most promising candidate for future development was sodium trans-[$RuCl_4$(DMSO)(HIm)] (NAMI, Novel Anti-Tumor Metastasis Inhibitor).[12] *In vivo* studies on a MCa mammary carcinoma xenograft model showed specificity for reduction of lung metastases and no effect on the growth of the primary tumor. The lifespan of the mice was significantly prolonged, particularly in combination with surgical removal of the tumor.[13] Finally, imidazolium trans-[$RuCl_4$(DMSO)(HIm)] (NAMI-A) (**Figure 1**) showed higher stability in air than NAMI but similar pharmacological effects.[14] NAMI-A was the first Ru-based anticancer agent to enter clinical trials and phase I studies were completed in 2004.[15]

During the mid-1990s, attempts were made to directly make Ru^{II} anticancer agents as this is the active form of ruthenium drugs and do require an activation step. However, many of these attempts were unsuccessful due to the lability of the complexes.[16-18] It was later found that the introduction of η^6-arene moiety greatly stabilize Ru^{II}.[19] In 2001, Dyson introduced [$RuCl_2$(cym)(pta)] (RAPTA-C) (**Figure 1**) as a metallodrug which showed DNA degradations at pH lower than 6.5 and good solubility in water.[20] Activity at a low pH meant that it could be used specifically to target diseased tissue such as hypoxic tumor tissue. Similarly to NAMI-A, *in vivo* studies in Mca mammary carcinoma xenografts showed that RAPTA-C reduced the growth of lung metastases but did not affect the primary tumor.[21] The mode of action of the RAPTA family was studied using Ehrlich's ascite carcinoma (EAC) model both *in vitro* and *in vivo.* Intraperitoneal administration of 40 mg/kg per week led to reduction in tumor growth by 50%.[22] It was found that DNA damage is likely not the only mode of action of this drug. Crystallographic studies showed that RAPTA-C forms specific adducts to the nucleosome core particle (NCP), specifically the the histone proteins. Therefore, the mode of action is thought to involve proteins rather than DNA in contrast to platinum metallodrugs.[22]

Finally, in 2001 Sadler introduced a novel type of Ru^{II} anticancer agents.[23,24] The strategy of coordinating a bidentate 1,2-ethylenediamine (en) moiety to the complex yielded highly antiproliferative effects against human ovarian cancer. In particular, [(η^6-bip)RuCl(en)]PF_6 (RM175) (**Figure 1**) showed antiproliferative activity comparable to carboplatin.[25] RM175 contains one halido leaving group and is believed to follow a different mode of action than the previously established Ru anticancer agents, by only binding monofunctionally to DNA.[26]

In conclusion, ruthenium-based anticancer agents have shown great promise in reducing or eliminating cancer without as many of the side effects as the platinum-based drugs of which they initially took inspiration. Ru^{III} drugs which follow a similar mode of action as cisplatin have shown value in and of themselves working on cancers to which cisplatin has no effect. On the other hand, Ru^{II} drugs have shown interesting modes of action, which diverge entirely from cisplatin altogether. However, mechanism of action of these compounds are yet to be fully understood. Understanding these will likely lead the development of better anticancer agents in the future.

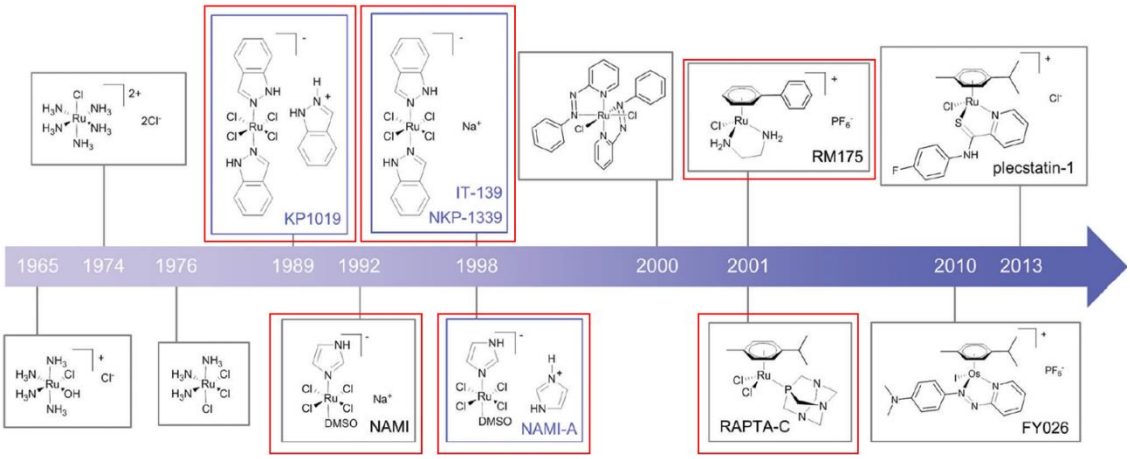

Fig. 1 Timeline of the discoveries of ruthenium and osmium lead structures. The highlighted purple compounds progressed to clinical studies.[28]

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